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Cover photo: Scanning electron micrograph of a shell of *Punctum* sp., a minute land snail (just about 1 mm in diameter) in the family Punctidae, a new record from Sonora, Mexico, one of the several terrestrial mollusks found by Van Devender *et al.* (pp. 315–322). SEM micrographs courtesy of R. Van Devender.

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Pyramidellid protoconchs, eggs, embryos and larval ecology: an introductory survey

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Abstract: Major papers on pyramidellid protoconchs, larval development, and larval ecology are reviewed, partly with illustrations. Larval shell coiling (including hyperstrophy and heterostrophy) is discussed. A-, B-, and C-type protoconchs are figured and distinguished. Those of species in the Baltic and northwestern France have been used to infer modes of larval development. The reliability of many of these inferences is questioned. Egg, embryo, and veliger morphologies, functions and terminologies are then discussed, including confusions of the terms "egg" and "capsule." The pigmented mantle organ or gland ("PMO") is briefly reviewed. Previously published data on the eggs and larvae of *Brachystomia "scalaris"* (Macgillivray, 1843), of "*Turbonilla*" *cummingi* Hori and Okutani, 1997, and of "*Odostomia columbiana* Dall and Bartsch, 1907", are reviewed in detail with some new interpretations. In the first of these species, egg size variation is possibly salinity-induced and may cause variation in mode of development. Alternatively, different species may be involved. In the second named species, lecithotrophy straddles intracapsular metamorphosis sympatrically. In the third species, some of its unusual features may have been induced by low temperatures. "Mid-water metamorphosis" is then discussed. It is concluded that species with A-type protoconchs may be constrained to planktotrophy.

Key words: A-, B-, and C-type protoconchs, embryonic development, larval development, veligers

This paper reviews general background information needed for understanding the discoveries reported in the next two papers.

Many pyramidellid larval shells and protoconchs are left-handedly coiled and appear sinistral. In actuality they are dextral and hyperstrophic. The direction of coiling around the axis remains the same but the polarity is reversed. The original base of the shell becomes a false spire and *vice versa.* Pelseneer (1914: 8), Lebour (1932), and Rasmussen (1944: 214) all observed anatomically that pyramidellid veligers are hyperstrophic. Sinistrality cannot switch to dextrality at metamorphosis. Isostrophy is another term for planispirality (discoidal coiling). Heterostrophy is here defined as larval hyperstrophy; it is not solely a tilted protoconch (common with heterostrophy). No adult pyramidellids are hyperstrophic. Gastropod coiling is further discussed and illustrated by Robertson (1993, 2003).

Although they intergrade, pyramidellid protoconchs can be classified into three main kinds. Fairly consistent illustrations and descriptions of them are by Aartsen (1987a), Linden and Eikenboom (1992: 4, 5, figs. 2–7), Schander (1994: 13), Peñas *et al.* (1996: 8–9), and Peñas and Rolán (2010: 19–22, figs. 1–2). Figure 1 shows three representative images from Peñas *et al.* (1996), with Fig. 1A showing an A-type protoconch, Fig. 1B a B-type, and Fig. 1C a C-type. A-type protoconchs have 2+ heterostrophic whorls, and their coiling axes are about at right angles to those of the teleoconchs. B-type protoconchs consist of 1½ to 1¼ somewhat heterostrophic or nearly isostrophic whorls, and are somewhat less tilted. C-type protoconchs consist of about 1 isostrophic whorl, and this shows no external heterostrophy or tilting. It is somewhat surrounded by the first teleoconch whorl, and is not overturned 180°. The position of non-A larval shells in teleoconchs was shown semi-diagrammatically by Robertson and Orr (1961: fig. 6) and Minichev (1971: fig. E). No pyramidellid has as many protoconch whorls "submerged" or "intucked" in the early teleoconch as indicated by Fretter *et al.* (1986: fig. 377 A). Rodríguez Babío and Thiriot-Quiévreux (1975) published scanning electron micrographs showing four (differently defined) protoconch types in different views. Selecting the best of these: their pl. 1 figs. B, C, E, F, I and K, and pl. 2, figs. E and F all show A-types. Their pl. 2 figs. A–C and H all show B-types. Their pl. 1 fig. M, pl. 2 figs. I and L, pl. 3 figs. B, I and L, and pl. 4, fig. K all show C-types.

Previous studies

The animals are cross-fertilizing simultaneous hermaphrodites (Pelseneer 1899: 77, pl. 24, fig. 204), possibly with a trace of protandry. In consequence, they and their egg masses sometimes occur in pairs (Pelseneer 1914: 5), although I have not seen this. I have observed mating behavior and spermatophore transfer. The animals possibly use a sense of touch to detect conspecific shells, but this is probably not provable. The eggs and larvae are "opisthobranch-", not prosobranch-like

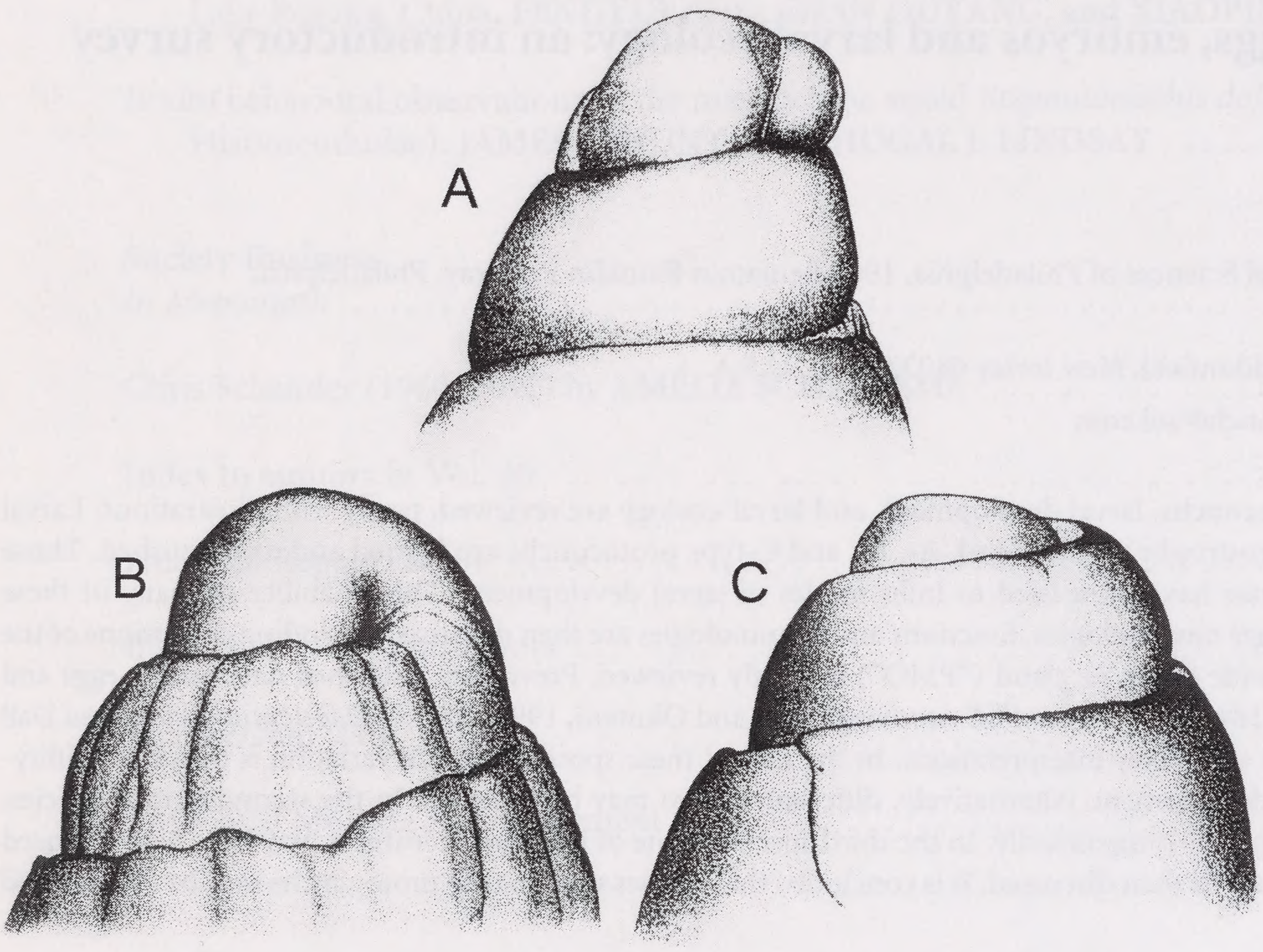

Figure 1. A–C, respectively, A-, B- and C-type pyramidellid protoconchs, which intergrade. All from Peñas *et al.* (1996: figs. 2 B, D and G).

(Thorson 1946). Hadfield and Switzer-Dunlap (1984: 235–236, 249, 271, figs. 12 A–B, 24 C) have summarized modern knowledge of pyramidellid reproduction. Previous world studies of pyramidellid larval development have been listed by White *et al.* (1985: table 8) and Collin and Wise (1997: table 1). Collin (2000) studied two more species.

Eggs and larval morphology, functions and terminology

The eggs are laid in gelatinous egg masses that are either nearly circular in outline (Fig. 2) or irregular, not externally structured as they are in most higher "opisthobranchs" (Hurst 1967; Soliman 1987; Klussmann-Kolb and Wägele 2001). As defined here, an egg is a fertilized ovum or zygote contained in an internal egg capsule. By "egg" authors such as Rasmussen, Thorson, White *et al.* and Cumming often meant an egg plus the surrounding capsule – by analogy with a chicken egg. "Egg" measurements based thereon are therefore misleading. The egg alone was sometimes also called the "yolk." Thompson (1972) appears to have been the first to use the term "capsule" for an internal structure in "opisthobranch" egg masses. Prosobranch egg capsules are external structures.

Capsules per egg mass range from an average of 50 (Pelseneer 1914) up to a total of 528 (Rasmussen 1944: 211). Maximum known capsule ("egg") length is 650 μm (Rasmussen 1951: 214). Capsules appear to swell slightly during development as they did more markedly in the nudibranchs studied by Kress (1972). The capsule walls are 1- or 2-layered, and their structures and ultrastructures are likely to be similar to those of higher "opisthobranchs" studied by Eyster (1986) and Klussmann-Kolb and Wägele (2001). Capsules are oval or elliptical in outline. They were called "envelopes" by Lebour (1932) and "cocoons" by Thorson (1946: 206) and others.

Chalazae are the threads within the egg mass connecting the capsules end to end in the sequence in which they were laid (Figs. 3–4). They are easily overlooked, which may explain why Høisæter (1989: 292) and others did not see them. Minichev (1971: 224. fig. Б) illustrated chalazae that apparently did not connect the capsules: at one end they did not extend beyond the capsule wall, and at the other end they were initially wide but tapered to fine points. There usually is one egg per capsule. Rarely, there are two (Pelseneer 1914: 6), two that are connected (Rasmussen 1951: fig. 6 b), or in one instance some capsules contained two or three embryos (Minichev 1971: 223). Lebour (1936: 556, pl. 2, fig. 11) reported four to eight eggs per capsule, but she could have seen 4- to 8-celled embryos. Eggs can be missing from alternating capsules (Fig. 5), but I have not seen this.

The egg diameter range is about 50 μm up to 340 μm (maximum length of oval eggs: LaFollette 1979). Below about 90 μm they are planktotrophic. Pelseneer (1914: 6, pl. 2, fig. 1, gl. p.) reported embryos generally with 3 polar bodies, and Collin and Wise (1997: 247, 248) apparently also saw them. I have not. Paired larval kidneys were reported only by Pelseneer (1914: 7–8, pl. 2, fig. 12, r. l.). Conspicuous paired statocysts and a stomach with bilobed digestive glands appear early, then small paired velar lobes with subvela, then opercula and bristles at the tip of the foot, then paired eyes, and tentacles usually last in larval development.

The term "pigmented mantle organ" (PMO), proposed by Robertson (1985), is perhaps more properly a gland. It is conspicuous near the anus in most larval (and adult) pyramidellids and other lower "opisthobranchs." The pigment is finely particulate, highly variable in color, and its chemical composition is still unknown. Functionally, it may be repugnatorial. It was called an anal gland by Pelseneer (1914: pl. 2, figs. 11–12, gl. a.), and has also been called a larval kidney (Schaefer 1996: table 21.1). He preferred to call it an anal gland but it is not what currently is called that in neogastropods (*e. g.* Andrews 1992).

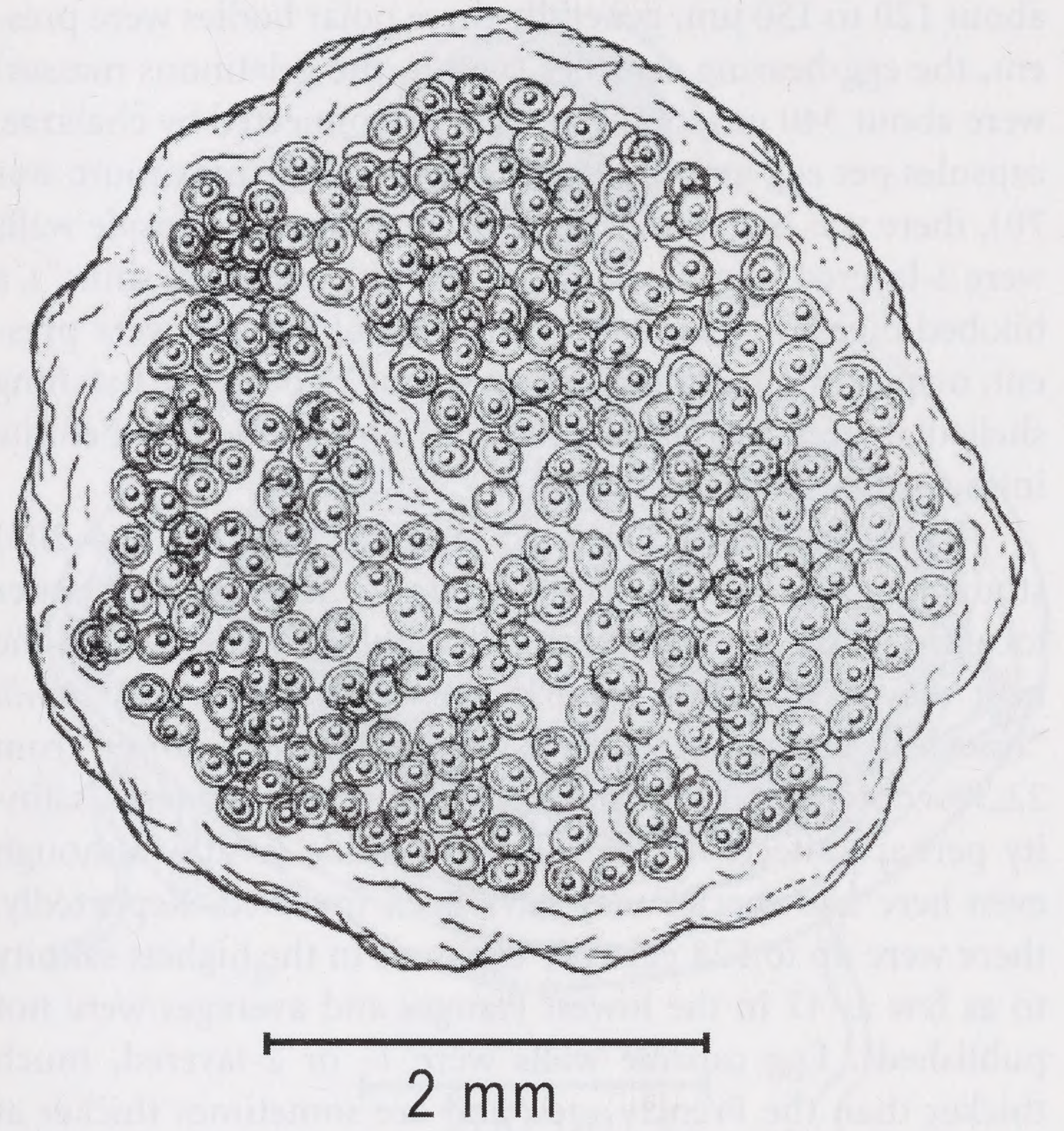

Figure 2. *Brachystomia* "*scalaris.*" Eastern Denmark. Gelatinous egg mass. Note that the egg capsules are not partly linearly arranged as they are in *Boonea*. From Rasmussen (1944: fig. 3, as *B.* "*rissoides*").

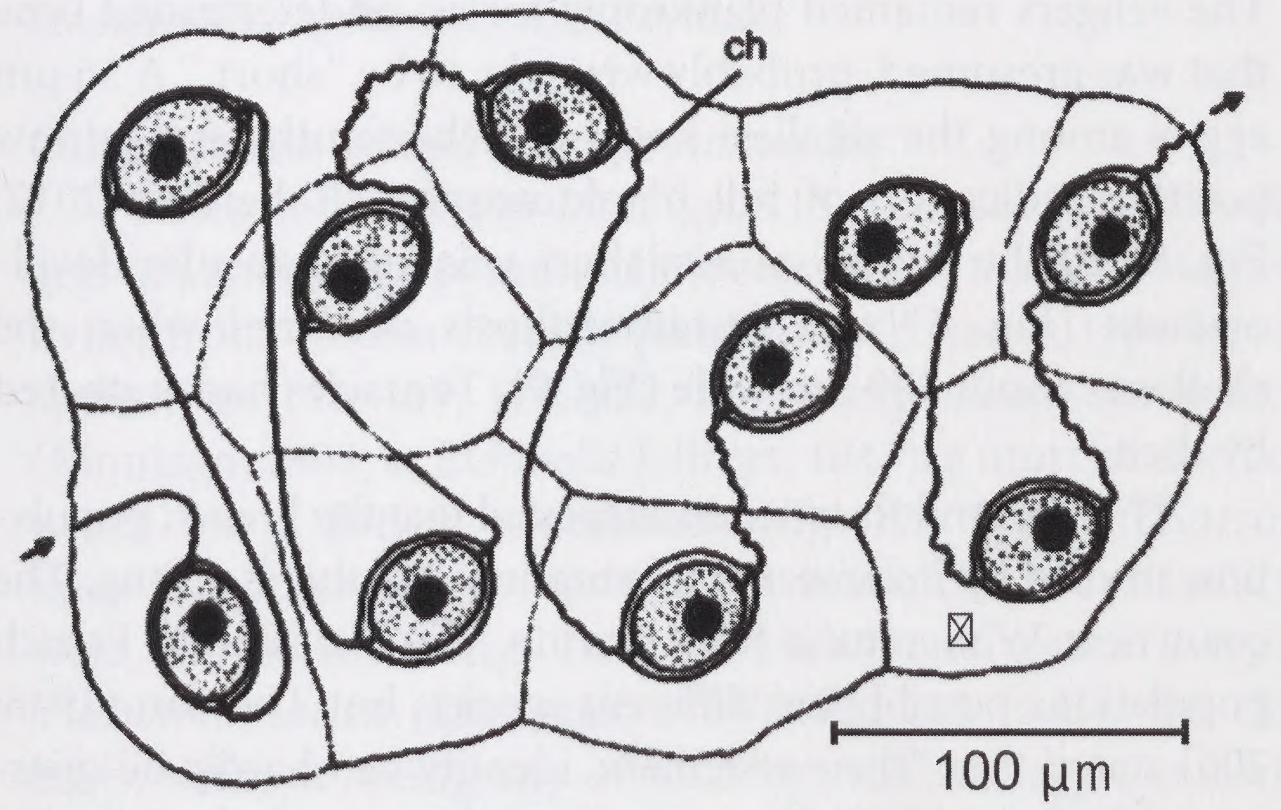

Figure 3. *Brachystomia* "*scalaris.*" Northern France. Part of egg mass showing contained egg capsules connected by chalazae (**ch**). The two arrows show the sequence of capsule formation. The compartments around each capsule are schematic. From Pelseneer (1914: pl. 1 fig. 4, as "*O. rissoides*").

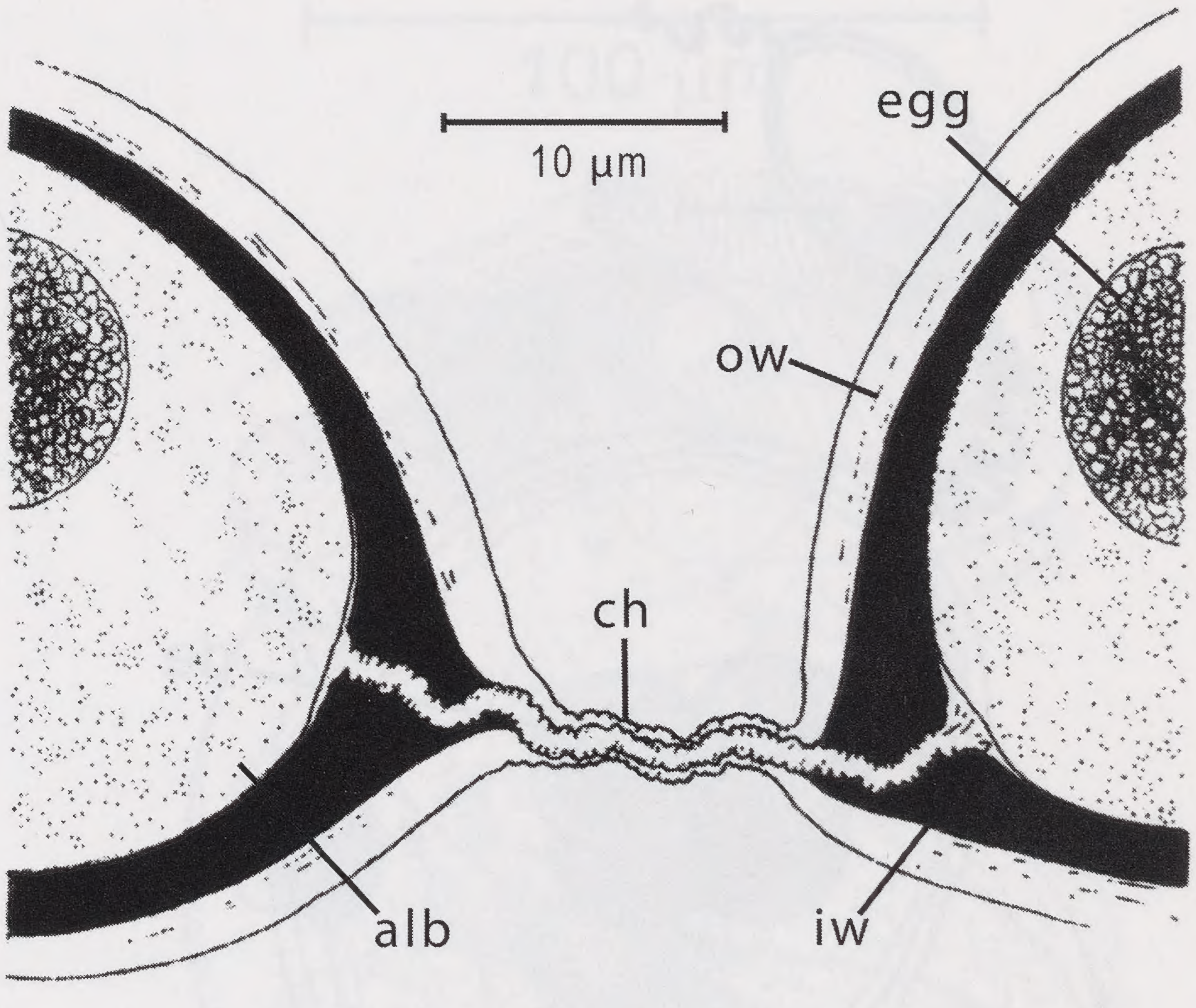

Figure 4. *Brachystomia* "*scalaris.*" Northern France. Close-up of parts of two egg capsules, their connecting chalaza (**ch**), parts of their contained eggs, the surrounding albumen (**alb**), the outer capsule wall (shown white, **ow**), and the inner capsule wall (shown black, **iw**). From Pelseneer (1914: pl. 1, fig. 5).

Intracapsular albumen surrounds the eggs, is granular, and disappears during development. As in nudibranchs (Eyster 1986), it probably is food for the developing embryos or pre-hatching early larvae. Extra-capsular yolk such as was reported in some sacoglossans and some nudibranchs by Boucher (1983) is not known in pyramidellids.

The larval shells are invariably coiled (shell-type 1: Thompson 1961). They show water repellency or hydrophobicity, the function of which is still obscure (White *et al.* 1985; Collin 1997). Misunderstanding the cause, Amio (1955) reported this in two other "opisthobranchs."

The name *Odostomia* Fleming, 1813

Pyramidellid shells with smooth teleoconchs are often grouped in the genus *Odostomia* without this being further characterized and no matter the provenances of the species. Such a group is likely to be polyphyletic. Indications of this are in the European species of "*Odostomia*" studied by Aartsen (1987a) because he illustrated (fig. 1, A–C) A-, B-. and C-type protoconchs all in this "genus." These usually characterize different genera. The type species, *O. plicata* (Montagu, 1803), has a B-type protoconch.

Brachystomia Monterosato, 1884 has similar teleoconchs, but the type species, *B. rissoides* (Hanley, 1844), has a C-type protoconch. Species mentioned in the present trio of papers that seem least likely to belong in the genus *Odostomia* are distinguished as "*Odostomia*", meaning *sensu lato*.

***Brachystomia* "*scalaris*"** (Macgillivray, 1843: 154–155) [type locality: eastern Scotland] and "*Odostomia*" *rissoides* [type locality: English Channel]; related species or synonyms?

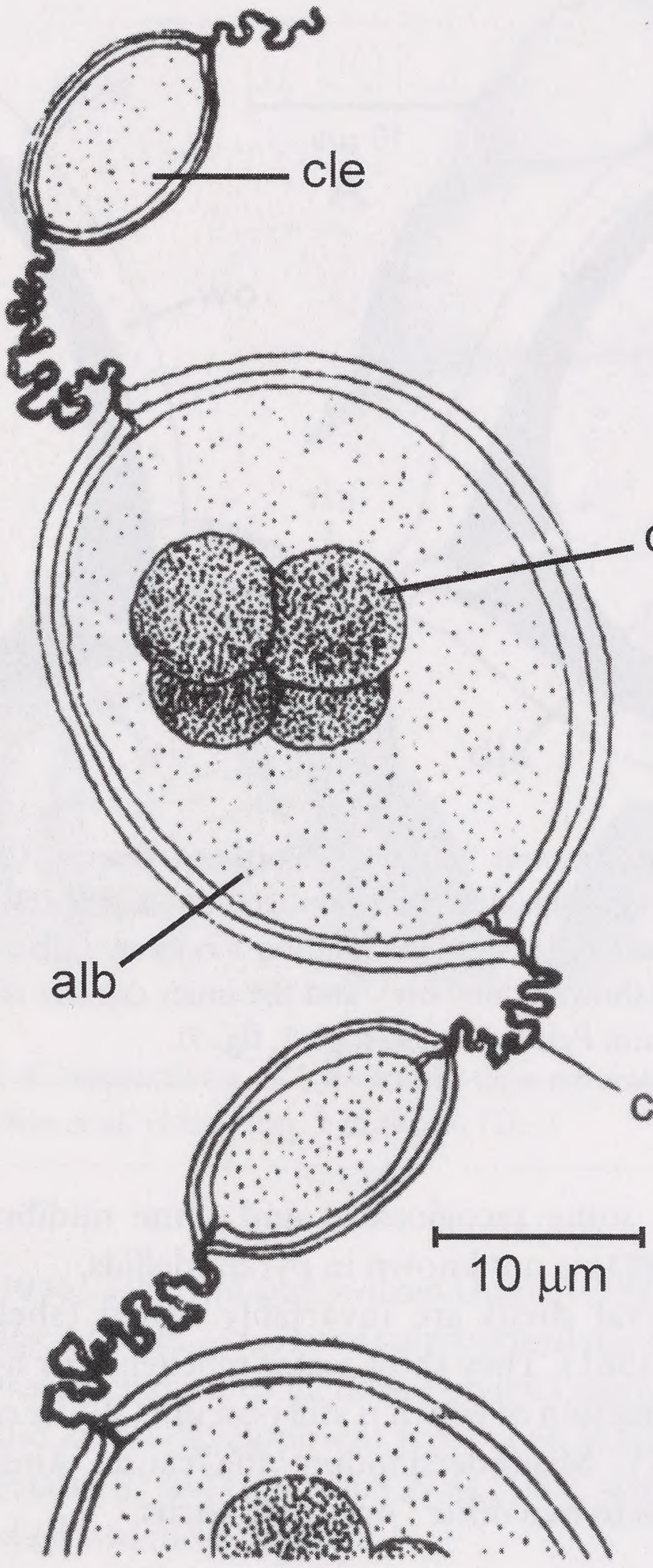

Figure 5. *Brachystomia* "*scalaris.*" Northern France. Part of an egg mass with alternating capsules lacking eggs (**cle**) and containing developing embryos (**de**); the capsules all contain albumen (**alb**); chalazae (**ch**) connect all the capsules. From Pelseneer (1914, pl. 1, fig. 6).

This was the first pyramidellid to have its development studied (Meyer and Möbius (1872: pl. opposite p. 20 + 65, 66). Aartsen (1987a: 12; 1987b) synonymized the two names on the basis of date priority, but probably more than one species is involved (hence the consistent use here of quotation marks for "*scalaris*", *i. e. sensu lato*). It was long believed to be host-specific to *Mytilus edulis* Linnaeus, 1758 in France, Britain (Marshall 1899: 227), and the Baltic.

Pelseneer (1914) studied the eggs, embryological, and larval development of "*Odostomia rissoides*" in northern France (Figs. 3–5), near Boulogne-sur-Mer and at and to the south of Wimereux, Pas-de-Calais. The egg diameter was about 120 to 150 µm, generally three polar bodies were present, the egg-bearing capsules (within the gelatinous masses) were about 340 µm long and were all connected by chalazae, capsules per egg mass averaged above 50 (the maximum was 70), there was one, rarely two eggs in a capsule, capsule walls were 2-layered (Fig. 4, ow, iw), a heart ("contractile sinus"), a bilobed digestive gland and paired larval kidneys were present, oviposition to hatching took about 25 days, the hatching shell diameter was about 400 µm, and there was definite intracapsular metamorphosis.

Rasmussen (1944, 1951) and Thorson (1946: 199, 204–206) studied supposedly the same species at five brackish water localities in eastern Denmark and nearby Sweden, where the host was also *Mytilus edulis.* They called it *Brachystomia* "*rissoides.*" The protoconch was C-type. Salinities ranged from 22.5‰ down to 16.1‰, not a wide range. Nonetheless, salinity perhaps affects the reproductive mode greatly, although even here two species may have been involved. Reportedly, there were up to 528 eggs per egg mass in the highest salinity to as few as 47 in the lowest (ranges and averages were not published). Egg capsule walls were 1- or 2-layered, much thicker than the French ones, and are sometimes thicker at one end than the other. Egg masses (Fig. 2) differed slightly from those of *Boonea.* Capsule lengths ranged from 180 µm to 200 µm in the most saline waters and up to 585 µm in the most brackish water. Only in the latter, the capsule is unusually large relative to egg size. The ratio is 1.0 : 0.19; in the former the ratio is 1.0 : 0.35, approximating that in *Boonea* (1.0 : 0.32). Egg diameters ranged from 55 µm at the highest salinity and up to 120 µm where it was lowest (Fig. 6). At the highest salinity veligers hatched (Fig. 7A–B) 6½ days after oviposition. After a week of development, an operculum was observed, and after two weeks eyes (Rasmussen 1944: 213). The veligers remained planktonic for an undetermined time that was presumed, probably wrongly, to be "short." A 55 µm egg is among the smallest known in the family, and is now positive indication of full planktotrophy (Robertson 2012: Fig. 1). At the lowest salinity there was intracapsular development (Fig. 8) and metamorphosis occurred when the shell was about 300 µm wide (Fig. 9). Tentacles had appeared by then.

Thorson and Rasmussen suggested that the French population studied by Pelseneer was estuarine, but this is wrong. The coast near Wimereux is fully marine. The Danish and French populations possibly are different species, but Thorson (1946: 206) stated that "their systematic identity can hardly be questioned." He could not have compared the animals directly, and he wrongly assumed host-specificity. Ankel and Christensen (1963) and Rasmussen (1973: 255–257, both as "*Odostomia*") recorded numerous additional molluscan hosts. Aartsen (1987a: 12) called "*Odostomia scalaris* polymorphous." Even in Norway, Høisæter (1989: 290, 291, fig. 4) recognized two forms or

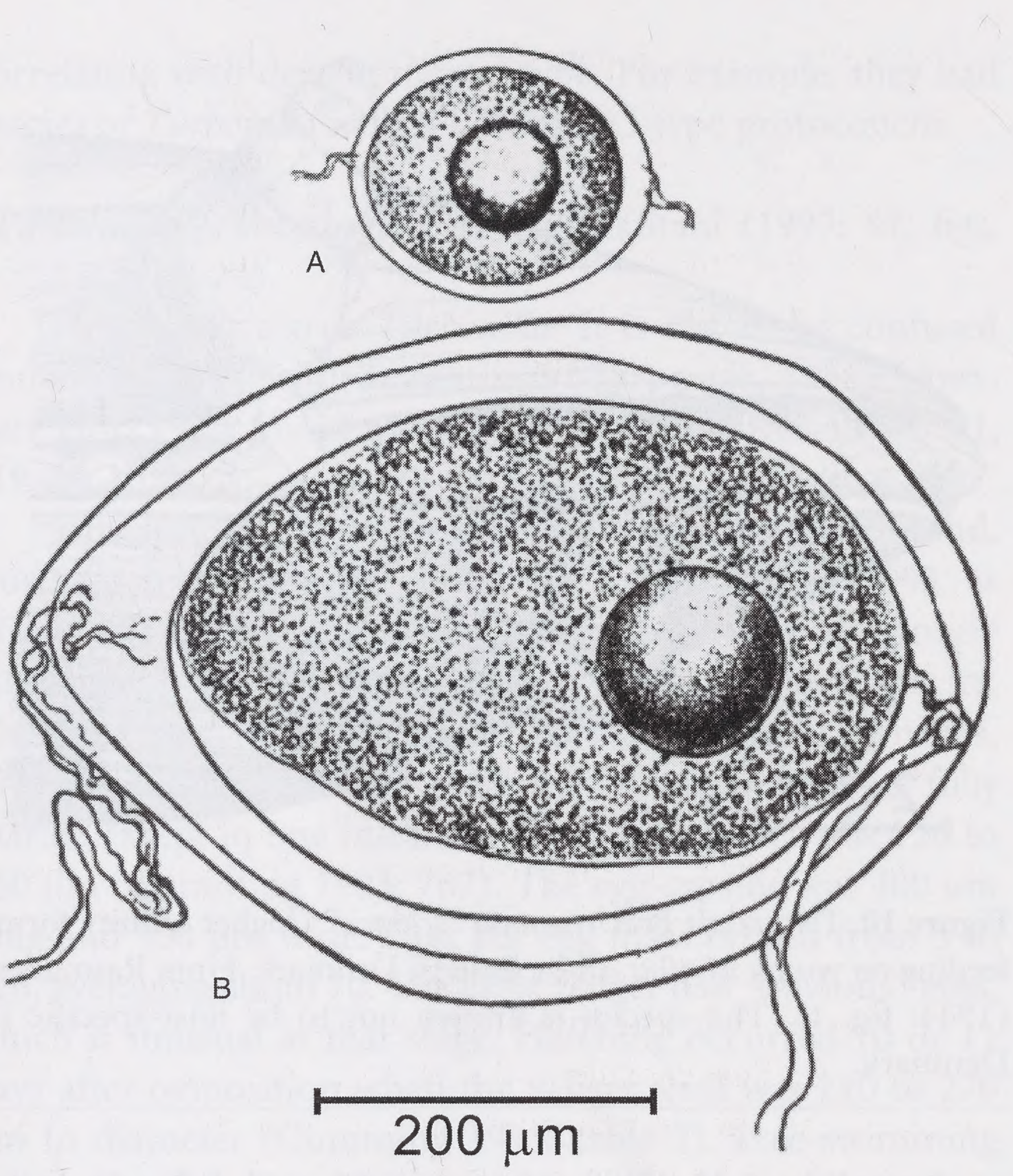

Figure 6. *Brachystomia* "*scalaris.*" Eastern Denmark. Egg capsules and eggs from **A**, slightly higher (19‰) and **B**, slightly lower brackish salinities (16.1‰), both at same scale. Possibly they are different species. From Rasmussen (1951: fig. 5).

possible sibling species of *Brachystomia* "*rissoides.*" He recorded (1989: 295) that neither of them were found there on *Mytilus edulis*, the commonest host elsewhere (Fig. 10). Mulder (1965) recorded *B.* "*scalaris*" living at the Netherlands in normal seawater salinities, apparently with *Mytilus.*

Other Danish veligers and protoconchs

Thorson (1946: 199–212, figs. 115–124) studied the veligers of about seven pyramidelloidean species that he obtained living from eastern Danish plankton. All had A-type larval shells (figs. 116–119, 121, 122, 124) except *Ebala nitidissima* (Montagu, 1803, as *Eulimella* Jeffreys, 1847), a murchisonellid, which has a B-type protoconch (fig. 123 D–G). Thorson knew five other species only from protoconchs, four of which were A-type ones (fig. 115 A–D) and one was a B-type, *Turbonilla lactea* (Linnaeus, 1758) (fig. 115 E–G). On this basis, without seeing any of their egg masses, eggs, or early developments, Thorson inferred that all these species are also planktotrophic. He assumed that the higher the number of larval shell whorls (up to about 2½) the higher the probability of planktotrophy. Thorson also illustrated the C-type protoconchs of eight more species (fig. 115 I–Q) and assumed on this basis alone that these have generally non-planktonic

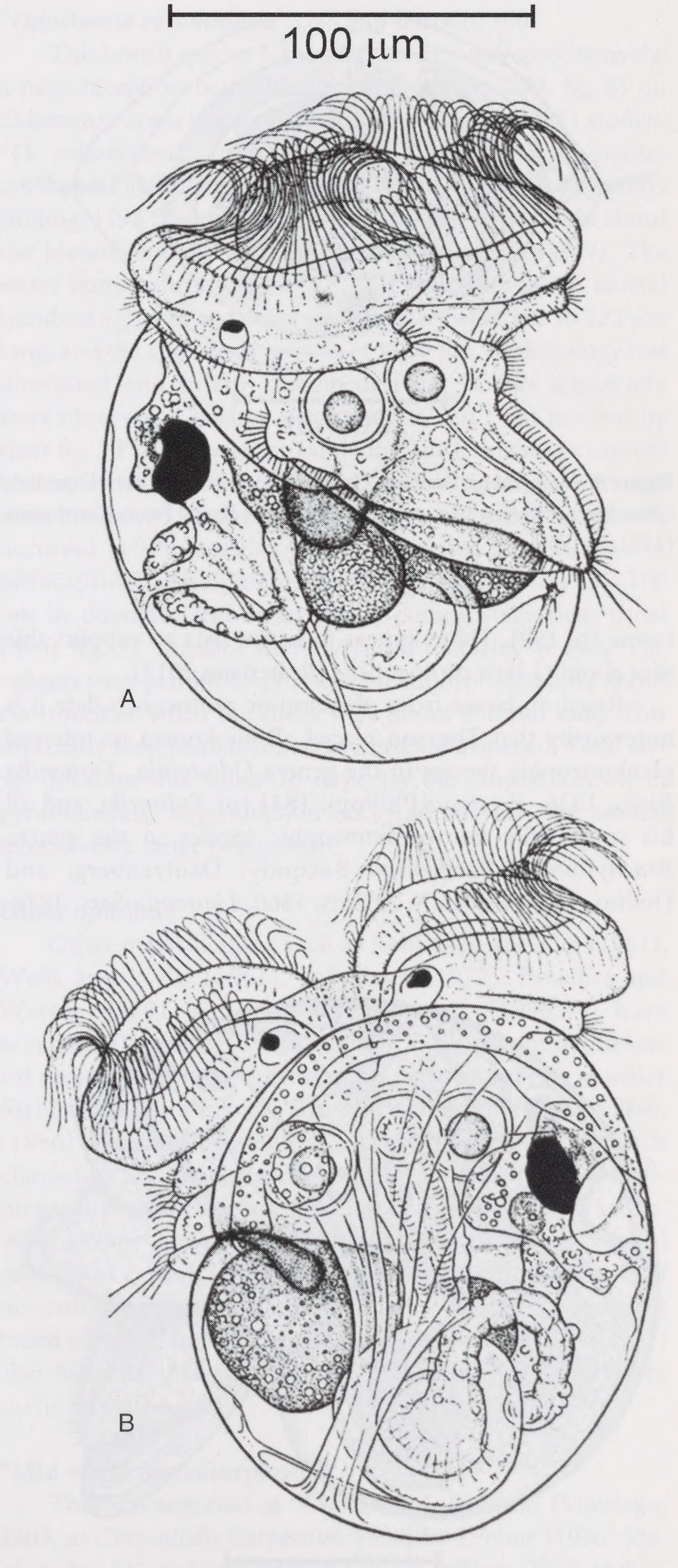

Figure 7. A–B, *Brachystomia* "*scalaris.*" Higher salinity form, eastern Denmark. Different views of a newly hatched veliger. The conspicuous dark and opaque gland is the pigmented mantle organ (**PMO**). From Rasmussen (1944: fig. 5).

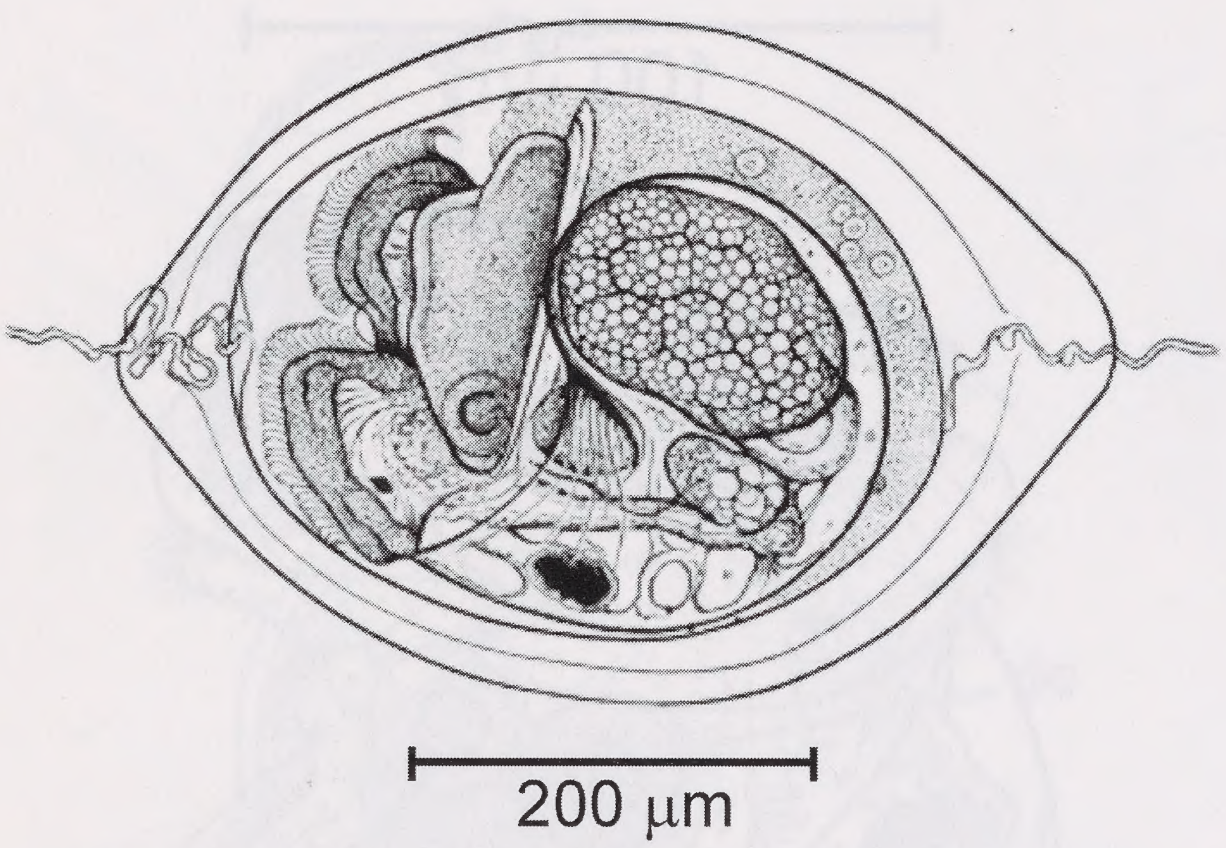

Figure 8. *Brachystomia* "*scalaris.*" Lower salinity form, eastern Denmark (possibly a different species). Intracapsular veliger. From Rasmussen (1951: fig. 7).

larvae (p. 199). There appear to be no data to support this idea about C-type protoconchs (Robertson 2012).

Based on larvae from plankton or protoconch data it is noteworthy that Thorson placed all his known or inferred planktotrophic species in the genera *Odostomia, Turbonilla* Risso, 1826, *Pyrgiscus* Philippi, 1841, or *Eulimella,* and all his supposedly non-planktotrophic species in the genera *Brachystomia, Parthenina* Bucquoy, Dautzenberg, and Dollfus, 1883, *Evalea* A. Adams, 1860, *Liostomia* Sars, 1878, or *Partulida* Schaufuss, 1869 (as "*Partidula*"). Only in *Brachystomia* "*scalaris*" did he observe alive in the laboratory development from the egg to the 4-celled stage (Robertson 2012: Fig. 1). Thorson presented only protoconch data about the second species of *Brachystomia* and the four other genera. He did not consider the possibility that sometimes pyramidellid protoconch types might reflect phylogeny more than they do larval ecology.

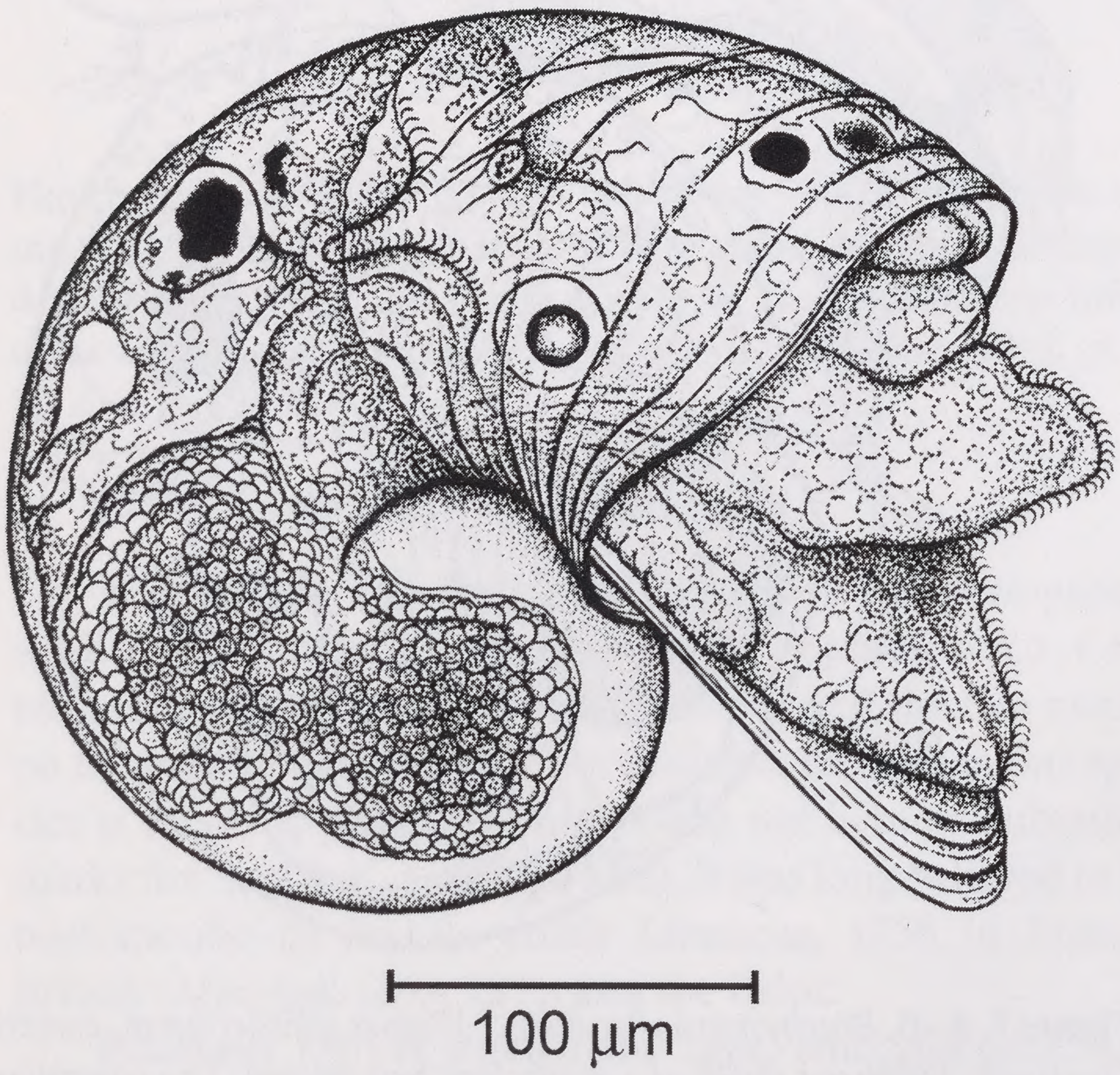

Figure 9. *Brachystomia* "*scalaris.*" Lower salinity form. eastern Denmark Young postlarva. From Rasmussen (1951: fig. 9).

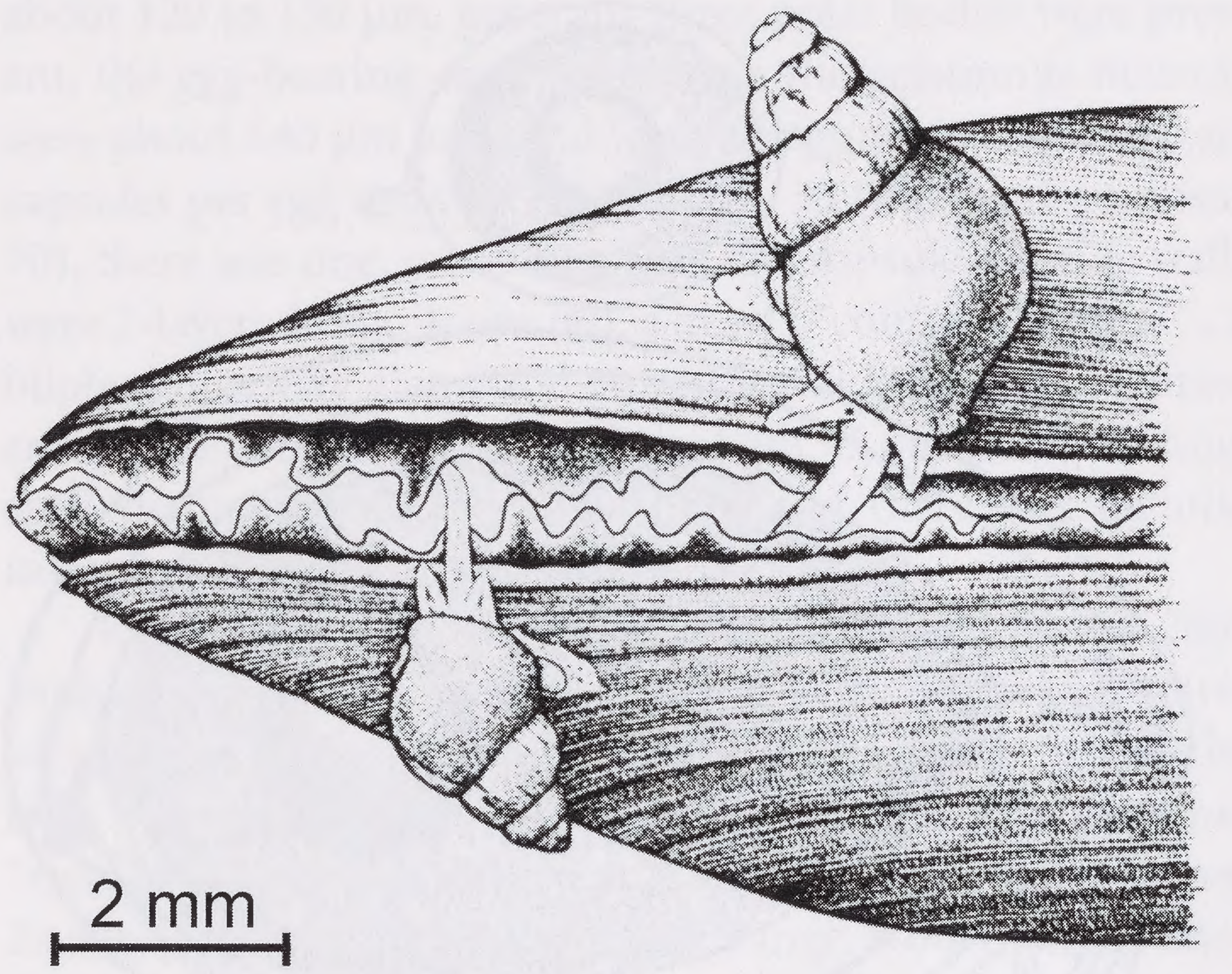

Figure 10. Two adult *Brachystomia* "*scalaris*" (higher salinity form) feeding on young *Mytilus edulis.* Eastern Denmark. From Rasmussen (1944: fig. 1). The species is known not to be host-specific in Denmark.

Northern French protoconchs

Rodríguez Babío and Thiriot-Quiévreux (1974, 1975) studied with a scanning electron microscope (SEM) the protoconchs of twenty-four pyramidellid and one murchisonellid species (*Ebala nitidissima*) from near Roscoff, Brittany, and inferred their larval ecologies without studying the living animals. They classified these protoconchs into 4-, not 3-types, and for some counted their whorl numbers and measured the embryonic and last whorls in lateral views. Largely following Thorson's (1946) criteria, they inferred that seven of their species, all with A-type protoconchs, have pelagic development. Three species had B-type protoconchs ("C-type" in their terminology), and, inconsistently, they thought that the *Ebala* and *Turbonilla crenata* (Brown, 1827) have pelagic development, and that *Eulimella* "*gracilis* Jeffreys, 1847" has a "direct" or short pelagic development. Fifteen species had C-type or approximately C-type protoconchs ("D-type" in their terminology), and they thought that, following Thorson (1946), all of them have a "direct" or short pelagic development. Just as with Thorson, there appear to be no data to support that idea. Their species did not sort into genera correlating with developmental type. For example, they had species of *Turbonilla* with A-, B-, and C-type protoconchs.

"***Turbonilla*** ***cummingi*** Hori and Okutani (1997: 81, figs. 2–13, 17–24).

This is not a true *Turbonilla.* It is not to be confused nomenclaturally with *T. cumingii* (Carpenter, 1856) [compare Hori 1995: 57–58, pl. 1, fig. 2; Peñas and Rolán 2010: 61, 419, fig. 155I].

This species was studied near Townsville, Queensland, Australia by Cumming (1988, as "*Pyrgiscus* sp."; 1993, as "*Turbonilla* sp."). Unfortunately, Cumming's work was poorly illustrated. Nonetheless, it is the best studied tropical species. The host was juvenile cultured *Tridacna gigas* (Linnaeus, 1758). No salinities were recorded but the water was fully marine except in one instance. The egg diameter was 120 to 150 µm (Cumming 1993: 767). The egg capsule was 400 µm long and 300 µm wide. Eggs per egg mass ranged from 3 to 226, averaging about 70. The early veliger had "obvious" eyes, which is unusual at that stage. Hatching occurred 10 or 11 days after oviposition when the veliger shell was 210 to 270 µm in diameter (Cumming 1993: table 1). Free-swimming veligers or crawling young emerged simultaneously, sometimes from the same egg mass. They came from embryos of the same sizes and appearance. The relative numbers of swimmers and crawlers depended on the degree to which an egg mass was agitated, water disturbance (at least in the laboratory) favoring swimmer release. These lost the capacity to swim 2 or 3 to 5 days later when the settling young shells ranged from 300 to 340 µm in diameter after velum loss. It is puzzling why there was this size increase while they were planktonic. This mode of development straddles sympatric lecithotrophy with intracapsular metamorphosis, which was otherwise unknown in pyramidellids. Davis (1967) and Carroll and Kempf (1990) made similar observations on two nudibranchs (*Phidiana* and *Berghia*), and Gibson and Chia (1995) on a cephalaspidean (*Haminoea*).

Cumming (1993: 766–767) recorded "*Turbonilla*" *cummingi* "eggs" half the normal size when the temperature had been extra high and salinity was low due to heavy rainfall. It is obvious from the measurements given that capsules were measured, not eggs. Lynch (1991 [unpublished]) studied this matter, believing that poecilogony was involved. Capsule size reduction would not necessarily lead to true egg size reduction. Population growth was remarkably rapid under culture conditions, and it is noteworthy that the lifespan is usually up to only 4 or 5 months (Cumming and Alford 1994: 109). With Boglio and Lucas (1997, also as "*Turbonilla* sp."), knowledge of the adult ecology of "*T.*" *cummingi* is second only to that on *Boonea* "*impressa*" (Say, 1822) among pyramidellids.

"***Odostomia columbiana***" Dall and Bartsch, 1907

This boreal species is the best one known reproductively, a near exception being Høisæter (1989: 292–293, fig. 5) on *Odostomia acuta* in Norway. Collin and Wise (1997) studied "*O. columbiana*" from San Juan Channel, Washington, northeast Pacific, where it parasitized two species of *Chlamys* Röding, 1798 (Pectinidae). They expressed some doubt about the identification of their "*Odostomia*" (pp. 248–249). The water temperature was 10–12° C. There were up to several hundred eggs per egg mass. Egg capsules were 193 to 222 µm long, and the egg size range was 71 to 77 µm. Embryology was illustrated and briefly described. Polar bodies apparently were observed. Chalazae were present, but I am puzzled by their fig. 2 F (a photograph) showing one traversing a capsule wall or interior. Prior to hatching, a beating larval heart was seen, but paired larval kidneys were not. Considerable growth occurred before hatching, which happened after 19 days of intracapsular development. The hatching shells were 150–160 µm in diameter and at an earlier coiling stage than usual (their fig. 3). Initially, there were no tentacles or eyes. The veligers were planktotrophic and metamorphosed after about two months, when the shells were about 290 µm long. This unusually long planktonic stage must aid dispersal. Total larval duration was about 70 days, far the longest known in pyramidellids. Reproduction occurred all year, the annual temperature range being slight.

Other opinions

Other malacologists, such as Robertson and Orr (1961), Wells and Wells (1961), LaFollette (1977), Bouchet and Warén (1979), and van Aartsen *et al.* (1998: 4) have accepted Thorson's (1946) inferences on pyramidellid larval ecology and have extended his ideas to species in other fossil and Recent marine and brackish faunas. Fretter *et al.* (1986) were ambivalent in accepting that protoconch characters are good indicators of modes of larval development, but that they are also good as generic characters. Wise (1996) agreed with Thorson in regard to the larval ecology of certain eastern American odostomians, but had no ontogenetic data and relied on a cladistic argument based on adult anatomy. Earlier authors such as Laws (1937) also believed that pyramidellid protoconch characters are useful in systematics.

"Mid-water metamorphosis"

This was reported in "*Odostomia*" *decussata* (Montagu, 1803, as *Chrysallida* Carpenter, 1856) by Lebour (1936: 556, pl. 2, fig. 14) and in *Odostomia acuta* Jeffreys, 1848 and *O. turrita* Hanley, 1844 by Thorson (1946: 202–204, 466–467, figs. 117 C–D, figs. 119 A–G; Fig. 11 here). Thorson illustrated *O. turrita* on the day of capture; their velar lobes were reduced.

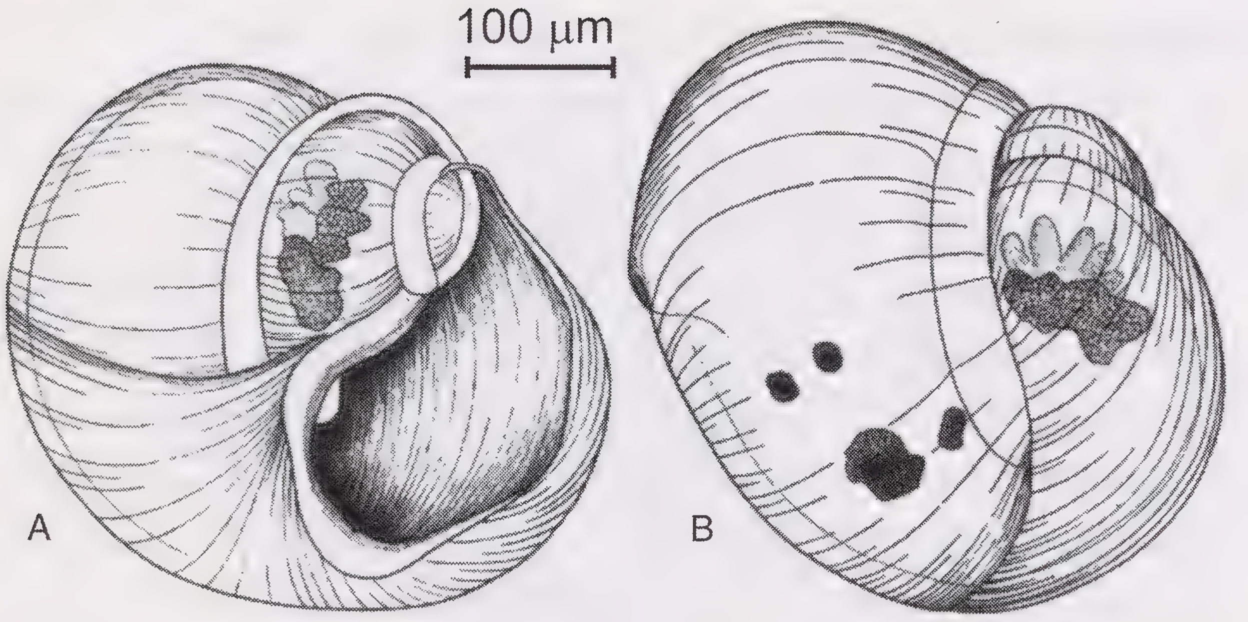

Figure 11. A–B, *Odostomia turrita* Hanley, 1844. Two views of shells at "mid-water metamorphosis." From Thorson (1946: fig. 119 F–G).

Conclusion: A-type protoconchs

As believed by Thorson (1946) and Rodríguez Babío and Thiriot-Quiévreux (1974, 1975), taxa with A-type protoconchs probably are constrained to planktotrophy. All of the about six pyramidellids that Thorson obtained as living veligers from Danish plankton were A-type species. But I have not been able to study any A-type species alive.

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B-type protoconchs and all three modes of larval development in eastern North American *Boonea* (Pyramidellidae)

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Abstract: The genus *Boonea* Robertson (1978), ectoparasitic on mollusks, consistently has a B-type protoconch morphology thought by Thorson (1946) to indicate planktotrophy in other pyramidellid genera. Studies in the laboratory of the eggs and larval development of *Boonea seminuda* (C. B. Adams, 1839), the type species, *B. bisuturalis* (Say, 1822), and *B. impressa* (Say, 1822) on the eastern North American seaboard confirmed planktotrophy. The egg diameter range there was 68 to 80 µm. *Boonea bisuturalis* had been reared through metamorphosis using algal cultures (Robertson 1967; Leibowitz 1979 [unpublished]). The developmental stages are newly illustrated here. *Boonea seminuda* and *B. bisuturalis* have different teleoconch sculptures, similar but different spermatophores, and are closely related. Northern *B. bisuturalis* and southern *B. impressa* are allopatric and hybridize or intergrade conchologically in a cline between Long Island Sound and Chesapeake Bay. One population of *B.* "*impressa*" had been found to be lecithotrophic on the Texas coast, at Port Aransas (White *et al.* 1985). A second Texan population, at Galveston, is shown here consistently to undergo intracapsular metamorphosis. The eggs were reared to hatching crawlers in the laboratory. The Galveston protoconch sizes and morphologies are the same as at North Carolina, but eggs from Galveston were much larger (212 to 236 µm in diameter). Durations of intracapsular development through metamorphosis and the pelagic larval stage in laboratory *B. bisuturalis* were virtually the same (respectively 15 to 20 and 16 to 20 days). In the three species, egg sizes greatly affect oviposition to hatching times, hatching sizes, and whether or not there is planktotrophy. Egg sizes do not affect oviposition to metamorphosis times, or protoconch sizes. North Carolinian and Texan *B. impressa* teleoconch sculptures and spermatophore sizes differ slightly. There could be a wide and continuous intraspecific range of conchological and/or reproductive variation, or allopatric poecilogony, or a chemically-induced ecotype, or (as seems most likely) one or more as-yet unnamed sibling species in the Gulf of Mexico. Regardless, this appears to be the first known exception to the "shell apex theory" (Thorson 1950). Everywhere, *B.* "*impressa*" is a serious pest of *Crassostrea virginica* (Gmelin, 1791). Some new data on *Boonea* systematics, adult ecology, and life history are presented. "*Boonea*" *scymnocelata* Pimenta *et al.* (2009), from Brazil, is not a *Boonea* because it has a C-type protoconch; its teleoconch is convergent with that of *B. seminuda*.

Key words: allopatrically variable development, exception to "shell apex theory"

"Shell apex theory"

The genus *Boonea* on the eastern North American and Gulf of Mexico coasts has uniform protoconch morphologies and the same wide range of sizes. Unexpectedly, it is not possible to ascertain from these their modes of larval development. This appears to be the first known case not to accord with Thorson's (1950: 33, fig. 6) "shell apex theory."

Thorson believed that closely related species have different protoconchs if their modes of larval development differ. From this it would follow that modes of development can be inferred from differing protoconch characters, these being influenced more by larval ecology than phylogeny (Thorson 1946, 1950). Since then, and especially after the advent of the scanning electron microscope (SEM) in the late 1960s (Robertson 1971), more and more paleontologists and neontologists have extrapolated kinds of larval development from protoconchs, *i.e.* whether or not species had or have planktotrophic, pelagic larvae (*e. g.* Shuto 1974; Robertson 1976; Jablonski and Lutz 1980, 1983). Systematic studies now routinely include such inferences (*e. g.* Leal 1991; Fortunato 2002). Already in 1946, Thorson had used pyramidellid larval shell characters to infer modes of development. The exceptions in *Boonea* have to be presented in the context of the incompletely known systematics and ecology of the taxa involved, hence the detailed treatment of these here.

Previous *Boonea* studies

The only previous studies of eastern American *Boonea* eggs, larval development, larval ecology and metamorphosis are those of Wells (1959), Leibowitz (1979 [unpublished]), and White *et al.* (1985). Wells (1959, as "*Odostomia*") observed the egg masses of *B. impressa* near Beaufort, North Carolina. He illustrated an egg mass, and, most importantly, a measured late embryo in its capsule. This clearly indicates planktotrophy.

The major study of *Boonea impressa* reproduction and larval development and life history in Texas by White *et al.* (1985, Fig. 1 A–F here) was based on egg masses obtained from animals on oysters at two localities near Port Aransas. They studied egg masses, egg sizes, embryology, intracapsular and hatched veligers, the short planktonic stage that they observed, and metamorphosis. Their main data are repeated here in Table 1, and they clearly show lecithotrophy. They gave contradictory information as to whether they also studied eggs from North Carolina, and claimed that there is lecithotrophy, not planktotrophy there. This and some of their other data are questioned later here.

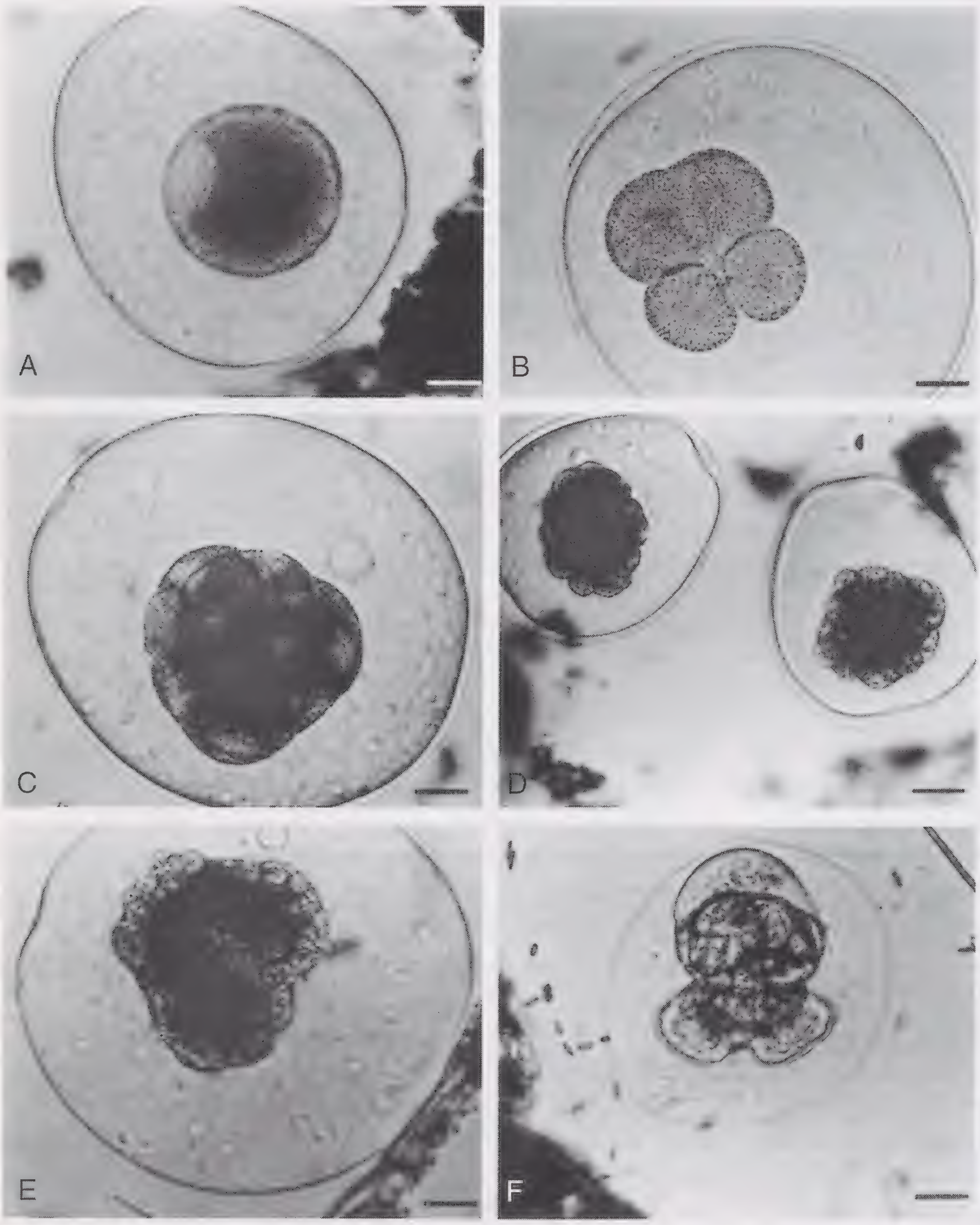

Figure 1. A–F, *Boonea* "*impressa*": developmental stages from egg to intracapsular veliger from a lecithotrophic population near Port Aransas, Texas. From White *et al.* (1985: 40–41, fig. 2). Scale bars: A, 40 μm. B, 24 μm. C, 23.6 μm. D, 57.5 μm. E, 21.5 μm. F, 38.8 μm.

Systematics and zoogeography

This study is based primarily on the common intertidal and shallow subtidal species of *Boonea* on the non-tropical eastern North American and Gulf of Mexico coasts. They are white-shelled or cream-colored and "*Odostomia*"-like. *Boonea* systematics and spermatophores were treated by Robertson (1978). The genus perhaps is unique among pyramidellids in having both a penis and spermatophores. The latter are consistently non-cuticularized and not shell-attached. (The spermatophore "attached" to the operculum of a *Boonea* would have been adhering loosely: Wise 2001.) *Boonea* may well be a synonym of some earlier-named taxon known only from shells, of which there are many candidates (Schander, Aartsen *et al.* 1999). (A leading contender is *Chrysallida* Carpenter, 1856.) Both Wise (1996) and Schander, Hori *et al.* (1999) studied *Boonea seminuda*, the type species of *Boonea*, in cladistic studies of pyramidellid morphology, yielding some clues as to its intrafamilial relationships. Rios (2009: 369) inconsistently and without explanation ranked *Boonea* as a subgenus of *Fargoa* Bartsch, 1955 but then continued to use *Boonea* generically.

Adult western Atlantic *Boonea* anatomy has been studied by Wise (1993; 1996: 488–489; 2001). Pimenta *et al.*'s (2009) figs. 1–14 (but not 15–20, see later) show the diversity in shell sculpture. They show divergent shell evolution well. Odé (1967) was perspicacious in recognizing that despite their greatly different teleoconchs, two *Boonea* species are closely related. *Boonea bisuturalis* (Say, 1822) is the only east American species with adults that have not yet been studied anatomically.

Pimenta *et al.* (2009) and Rosenberg (2010) have compiled the latest information on the systematics and zoogeography of the three *Boonea* species studied here. *Boonea seminuda* (C. B. Adams, 1839), the type species, ranges from Nova Scotia and Prince Edward Island, Canada, to Texas (Odé 1969) and Uruguay (Pimenta *et al.* 2009: 48; Brazilian teleoconchs: figs. 10–12). Its type locality is in Massachusetts, U.S.A., and its presence in the intervening West Indian tropics is somewhat uncertain

Table 1. Data from White *et al.* (1985) on major reproductive features of *Boonea* "*impressa*" near Port Aransas, Texas (see also Fig. 1 A–F here). It has B-type protoconchs. Ranges, means, and sample sizes (in parentheses).

Eggs per egg mass	about 20–250
Egg diameters (μm)	182–238[1] (54)
Oviposition to hatching (days)	"3.3–4.8"[2]
Hatching shell diameter (μm)	about 265[3] (1)
Veligers planktonic (days in lab)	< 7
Reproductive mode	Lecithotrophy

[1] The scale bar for Fig. 15 A wrongly indicates about 124 μm.
[2] Error. Did they mean time between hatching and metamorphosis?
[3] Estimated from fig. 3B.

(e. g. Redfern 2001: 143, B&W pl. 64, fig. 591). *Boonea bisuturalis* also ranges north to Canada and has a Massachusetts type locality (a photograph of its holotype first appeared in Pimenta *et al.* 2009: fig. 1). *Boonea bisuturalis* extends no farther south than the area between Long Island Sound and Chesapeake Bay. Here it hybridizes or its teleoconchs intergrade clinally with those of *B. impressa*, which has Maryland as its type locality. *Boonea trifida* (Totten, 1834), with a Rhode Island type locality, is conchologically intermediate (Winkley 1901; Scheltema 1965). Odé (1970) enumerated early Texan records of *B.* "*impressa*" and a *trifida*-like form sporadically occurs there also (Odé and Speers 1972: fig. 2). *Boonea* "*impressa*" apparently extends no farther south and east than to the southern Nichupté Lagoon, northeastern Yucatan Peninsula, Mexico, where shells were reported by Carnes (1975: 6, as "*Odostomia*"). Numerous records of *B. bisuturalis* in Texas are erroneous, and neither it nor *B.* "*impressa*" occurs in Brazil (Pimenta *et al.* 2009: 44). Bartsch (1909) published excellent illustrations of the teleoconchs of all these species, including *B. trifida* and other synonyms. His two records of *B. impressa* in Massachusetts (one from north of Cape Cod) presumably are erroneous. I have seen no definite New England specimens, although fossils have been recorded.

Boonea bisuturalis has been introduced into San Francisco Bay, California, where it apparently persists with (and probably feeds in part on) other introduced eastern American mollusks (Carlton 1979, Robertson and Mau-Lastovicka 1979, Nichols and Thompson 1985).

Because North Carolinian and Gulf of Mexico populations of *Boonea impressa* are disjunct and possibly distinct systematically, published illustrations of their shells are relevant. One from "Massachusetts" is in Bartsch (1909, pl. 13, fig. 51), a North Carolina one is in Bird (1970: fig. 8, no. 21), a "Florida" one is in Pimenta *et al.* (2009: fig. 2), two west Floridian ones are in Perry and Schwengel (1955: 122, pl. 23, fig. 162) and Gunderson (1997: 17), Texan ones are in Parker

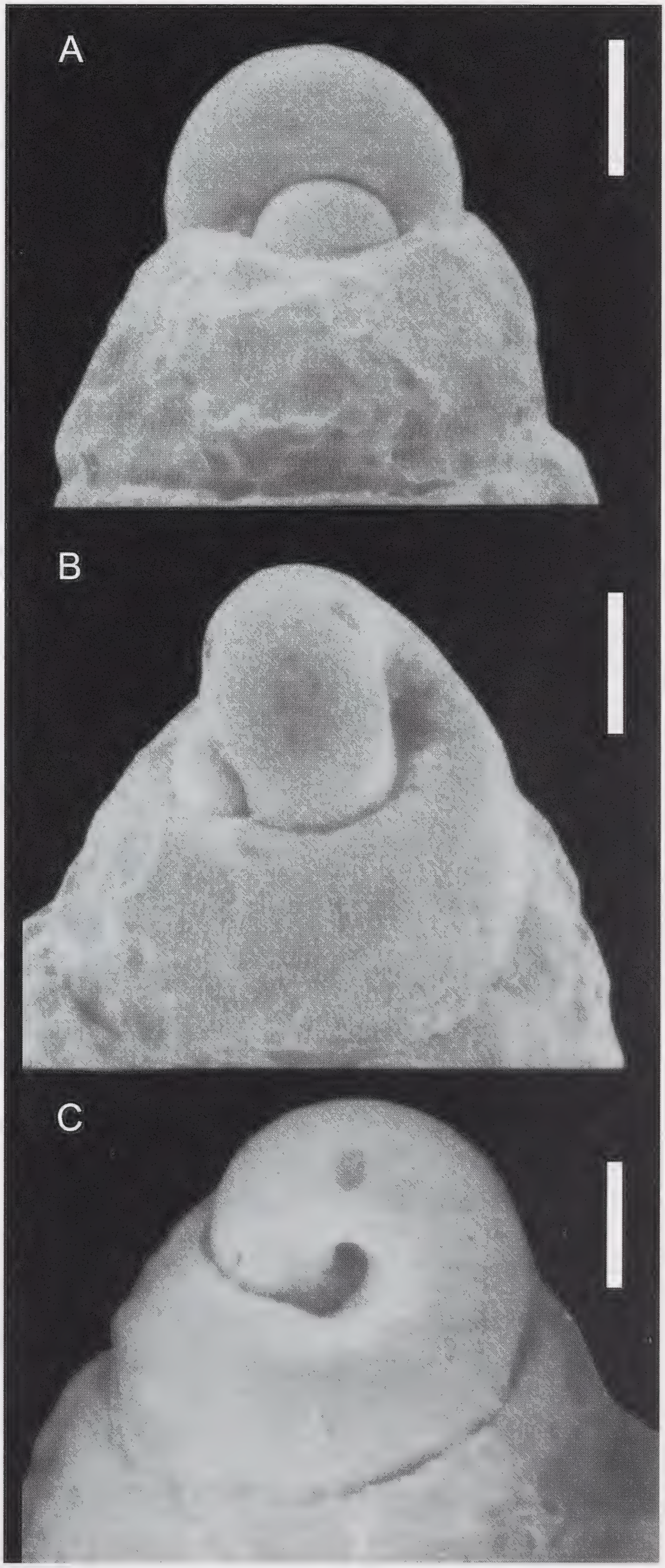

Figure 2. A–C, *Boonea seminuda,* the type species of *Boonea.* Three views of its B-type protoconch. Massachusetts. Planktotrophic. From Wise (1996: fig. 7 C–E). Scale bars: 100 μm.

(1959: pl. 1, fig. 21), Odé (1970), Andrews (1971: 130), Odé and Speers (1972: fig. 1), Robertson (1978: fig. 5), and Tunnell *et al.* (2010: 261), one from Laguna de la Mancha, Veracruz, Mexico is in Flores-Andolais *et al.* (1988: 240, pl. 2, fig. 19), a Campeche, Mexico one is in García-Cubas (1982: 75, 76, fig. 86), and, lastly, another from the southwestern to northeastern Yucatan Peninsula, Mexico, is in Vokes and Vokes (1983: 32, 59, pl. 30, fig. 10). *Boonea impressa* var. (?) *granatina* (Dall, 1884: 331), type locality "Cedar Keys" (northwest Florida), is a synonym of *B. seminuda*, not *B. impressa* (first synonymized by Dall, 1892: 251, as *Odontostomia* Jeffreys, 1839).

There are various published records of "Pliocene" and "Pleistocene" *B. seminuda* and *B. impressa* (including that of Clark 1906: 186–187, pl. 50, figs. 3–6, as *Odostomia*). According to Clark, a Miocene species is closely similar to *B. seminuda.* Bartsch (1955: 70, pl. 15, fig. 9) named the only possible Gulf of Mexico ancestor or form of *B.* "*impressa*": "*Chrysallida*" *woodringi.* This was from the "Pliocene" (Caloosahatchee Formation) of North St. Petersburg, west central Florida. It and the Upper and Lower Pinecrest beds are believed broadly to be Upper Pliocene or lowermost Pleistocene in age (Allmon *et al.* 1996: 274–275). *Boonea impressa* was recorded from the "Post-Pliocene" of South Carolina (Holmes 1858–1860, as *Odostomia*), and the Caloosahatchee ("Plio-Pleistocene") and Shell Creek of Florida (both Dall 1892: 251, as "*Odontostomia*"). A Pliocene bed at the Neuse River, North Carolina was made up almost exclusively of *B. seminuda* and *Crepidula fornicata* (Linnaeus, 1758), indicating that this host-parasite relationship (Robertson 1957) was already established by then (Porter 1976). This is the oldest fossil record of this *Boonea.* No fossil *B. bisuturalis* is known. Gulf of Mexico *B.* "*impressa*" (*sensu lato*) is here distinguished with quotation marks.

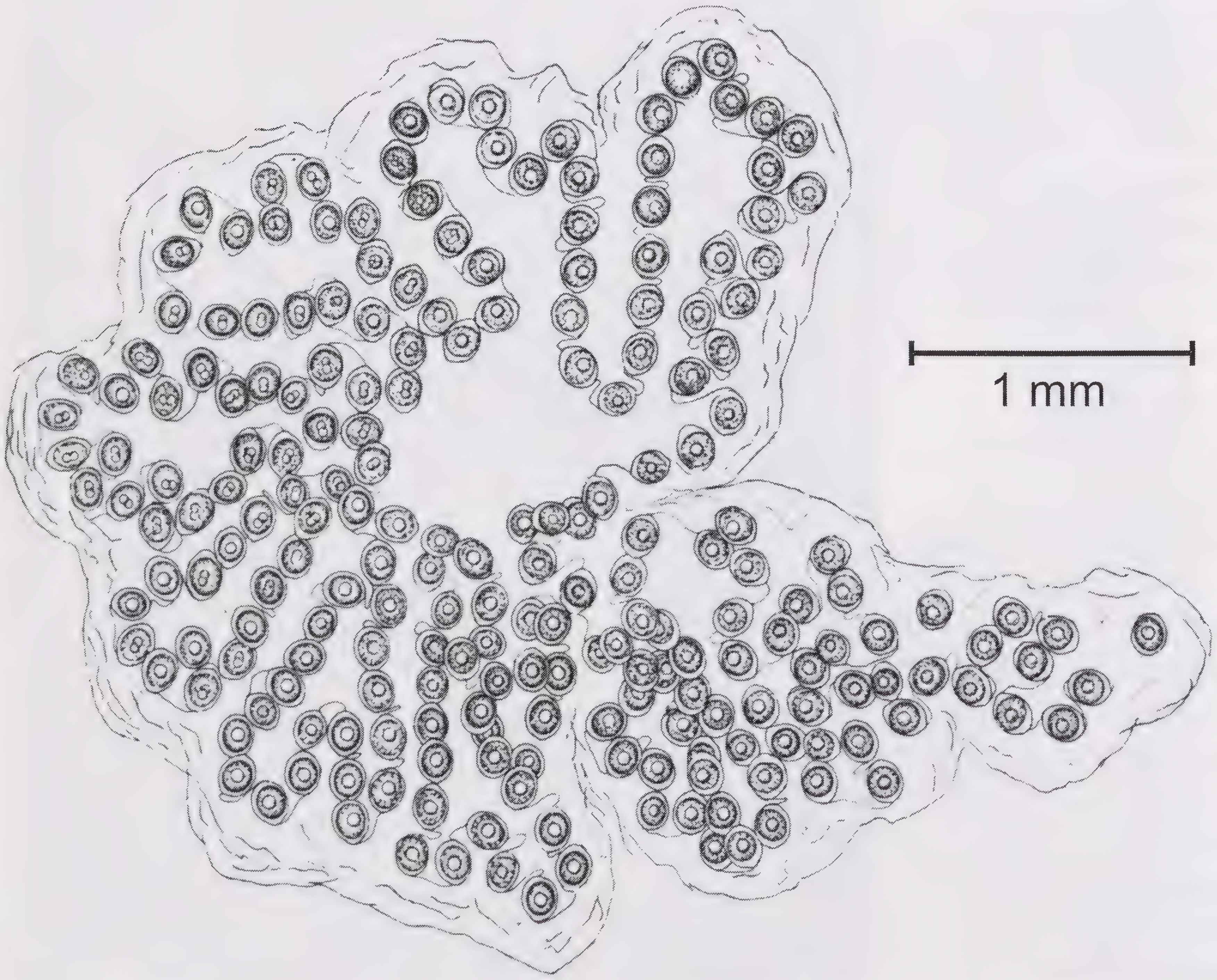

Figure 3. *Boonea seminuda.* Formless, gelatinous egg mass containing 216 eggs (the maximum observed), eggs and 2- and rarely 4-celled embryos each in an egg capsule. The chalazae are not evident at this magnification. Woods Hole. Planktotrophic.

North Florida range disjunctions

Boonea seminuda and *B. impressa* both have disjunct populations between the eastern North American seaboard and the Gulf of Mexico because neither occurs around southern peninsular Florida, which is tropical. On the east coast, these both occur as residents as far south as the Indian River Lagoon, Volusia and Palm Beach Counties (Mikkelsen *et al.* 1995: 114). This area is transitional between the subtropics and tropics. On the southwestern coast, *B.* "*impressa*" occurs as far south as Florida Bay, north of the Florida Keys, where William G. Lyons (unpubl. data) found it on *Ostrea equestris* Say, 1834, a new field host record. As long ago as 1892, Dall (p. 251) recognized that these two *Boonea* species have these disjunct distributions.

Other non-tropical organisms have similar distributions, relicts from the high sea stand in the Pliocene when the nontropical Atlantic and Gulf coasts were continuous (Avise 1992: fig. 2 A). The Suwannee Channel or Strait persisted until then. The Pleistocene interglacial shorelines were not as high. *Busycon perversum* (Linnaeus, 1758) is subspecifically distinct on the two coasts (Wise *et al.* 2004). The preferred adult host of *Boonea impressa, Crassostrea virginica* (Gmelin, 1791), is genetically distinct but not discriminated taxonomically (Hare and Avise 1996). *Littoraria irrorata* (Say, 1822) is unusual in apparently not being genetically distinct (Dayan and Dillon, 1995, as *Littorina* Férussac, 1822).

Boonea protoconchs

These seem uniformly to be B-type throughout the genus. Their morphologies and variable sizes in *Boonea seminuda* and *B. bisuturalis* closely

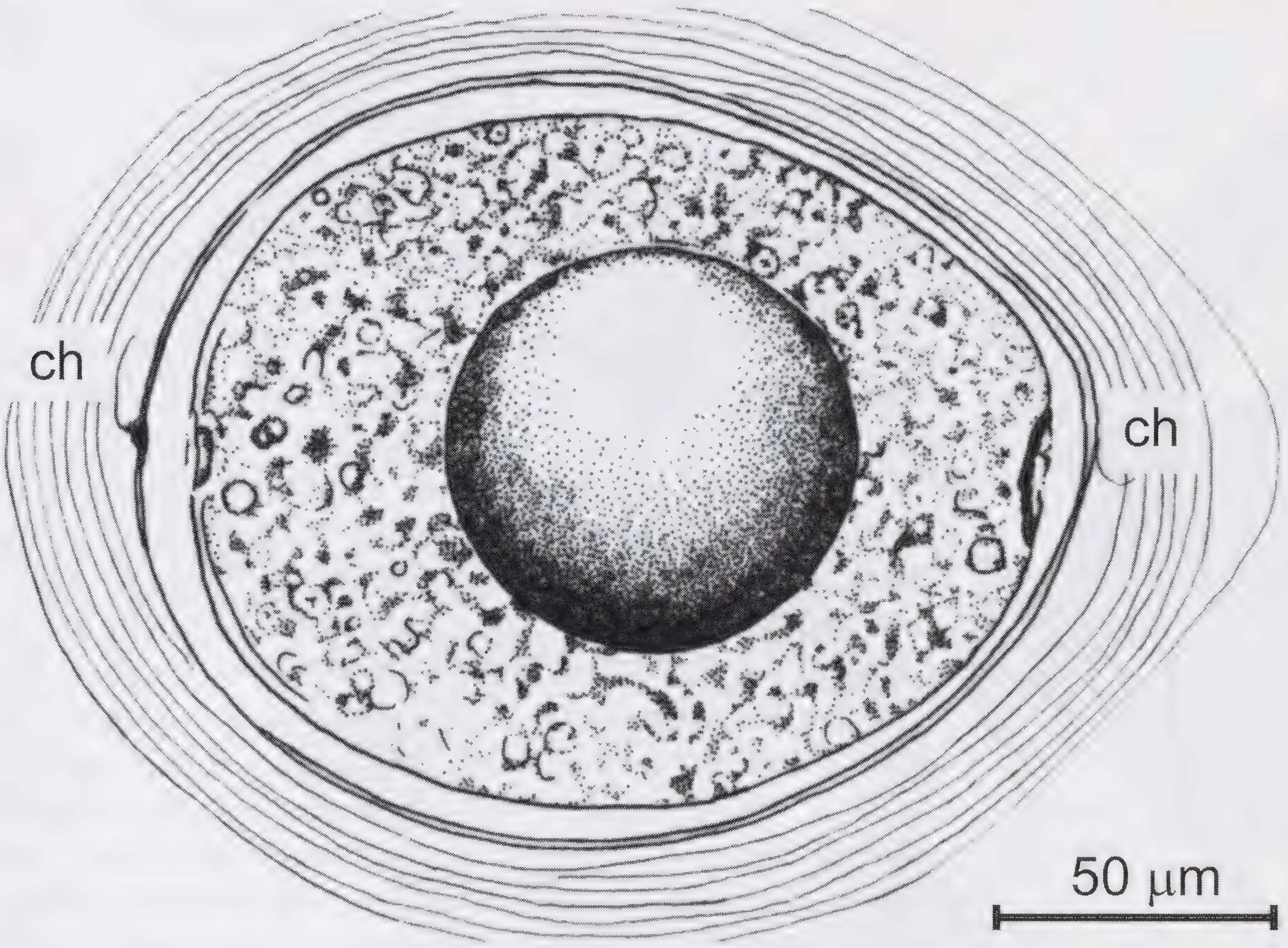

Figure 4. *Boonea bisuturalis.* An egg in its capsule, surrounded by mucous layers and with intracapsular albuminous granules. The capsule wall is not greatly thicker at one end than the other (as it is in *Fargoa*). Woods Hole. Abbreviation: **ch**, (fine) chalazae.

approximate those of non-Texan and Texan *B.* "*impressa*" (each is 200 to 295 μm in diameter). Those of *B. seminuda* from Massachusetts are re-illustrated here (Fig. 2 A–C). Wells and Wells (1961: fig. 7) also illustrated the protoconch of this species, showing that it differs from the C-type protoconchs of *Fargoa* (Robertson 2012b). *Boonea seminuda* has the same protoconch morphology in Brazil (Pimenta *et al.*, 2009: figs. 13, 14), so more new protoconch illustrations for this species seem unnecessary here. North Carolinian and Texan *B. impressa* larval shell widths were first compared by White *et al.* (1985: tables 4 and 5), who concluded that they have nearly the same size ranges. Their data accord closely with mine (240 μm versus 234 to 240 μm, *N* = 1086, respectively). New protoconch measurements for the three species are given here later in the Results section.

Adult *Boonea jadisi* (Olsson and McGinty, 1958), with a Caribbean Panama type locality, has been studied by Wise (2001) at Sebastian Inlet, east central Florida. There is no information on its hosts, eggs, or larvae, but Wise's figs. 3–5 and those of Pimenta *et al.* (2009, figs. 6–7) show that both in Florida and Brazil it has a B-type protoconch.

Boonea is not known in Europe, but it is native also in the Pacific. This has been determined primarily from adult anatomy. *Boonea cincta* (Carpenter, 1864) occurs in the eastern Pacific (Wise 1996: 489–491, fig. 9). It too has a B-type protoconch. Hori and co-authors have described four new species of *Boonea* from Japan: *B. umboniocola* Hori and Okutani (1995), *B. okamurai* Hori and Okutani (1996), *B. kinpana* Hori and Nakamura (1999), and *B. suoana* Hori and Nakamura (1999). All four have B-type protoconchs, and they each have different sole or preferred molluscan hosts. The protoconch of *B. omaensis* (Nomura, 1938), also Japanese, has not yet been studied.

"*Odostomia*" (*Evalea*) *somersi* Verrill and Bush (1900: 533, pl. 65, fig. 7) was transferred to *Boonea* by Odé (1993: 55) and Wise (2001: 74, as "*someri*"), but belongs in *Oscilla* A. Adams, 1861, according to Pimenta *et al.* (2008: 177, figs. 3 A–C).

Although it has teleoconch sculpture appearing closely similar to that of *Boonea seminuda* (the type species of *Boonea*), "*Boonea*" *scymnocelata* Pimenta *et al.* (2009: 47–49, figs. 15, 16, 20), from 175 m off Paraná State, southern Brazil, is not a *Boonea* because it has a C-type protoconch (their figs.17–19) that is not "greatly tilted" (compare their figs. 13 and 14 of *B. seminuda*). They mentioned this major protoconch difference. "*Boonea*" *scymnocelata* is of unknown generic affinities. This is an excellent example of evolutionary teleoconch convergence.

If Thorson's (1946) inferences are right, all B-type pyramidellid species and all true species of *Boonea* should consistently be planktotrophic. This is already known not to be true. *Boonea cincta* was shown by LaFollette (1979: 32, as *Chrysallida*) to have intracapsular metamorphosis and probably the largest known pyramidellid egg, which is 300 to 340 μm long and 270 μm wide (it is unusual in being oval). The capsule in which it is contained is 390 to 410 μm long and about 300 μm wide. *Boonea umboniocola* was shown by Nishino *et al.* (1983, as "*Odostomia* sp.") to be lecithotrophic. *Boonea okamurai* was shown by Hori and Okutani (1996) to have intracapsular metamorphosis. If it is truly a *Boonea*, *B. kinpana* has one of the larger pyramidellid protoconchs, 350 μm in diameter. It does not necessarily have intracapsular metamorphosis because *B. kinpana* could have an extra long planktotrophic stage.

Boonea adult ecology, abundance, and economic importance

Boonea lives on hard substrates and is ectoparasitic on mollusks. Their hosts and feeding were treated by Robertson

(1957) and Robertson and Mau-Lastovicka (1979). Robertson (2006) is a color-illustrated summary of the subject.

Where present, *Boonea* "*impressa*" is usually very abundant. Odé (1974) identified numerous mollusk specimens (mainly empty shells or bivalve valves) dredged from several Texas coastal bays, and kept detailed records of the numbers of each. From 21 stations in western Galveston Bay, he had 16,761 specimens of 121 species (20 of them pyramidellids) (his table 2, inserts A–F). His data on relative pyramidellid abundances are first analyzed here. "*Menestho impressa*" was the seventh most numerous species, with 565 specimens (3.4% of the total). Another pyramidellid, *Turbonilla interrupta* (Totten, 1835) [as *Pyrgiscus interruptus*], was fourth, with 982 specimens (5.9% of the total). *Turbonilla* is a soft substratum genus (Sanders 1958: 254, 255; 1960; Wharton 1976). Sanders was wrong in assuming that his species is necessarily host specific.

Both on the eastern North American seaboard and the coast of Texas, *Boonea impressa* is of economic importance because it is a serious pest on *Crassostrea virginica*. The extensive literature on its adult ecology is by Hopkins (1956), Wells (1959), Miller (1983), White *et al.* (1984, 1985), Ward and Langdon (1986), Powell *et al.* (1987a, 1987b), White *et al.* (1987, 1989), White, Powell *et al.* (1988), White, Powell, Ray *et al.* (1988), Wilson, Powell and Ray (1988, 1991), Wilson, White *et al.* (1988), and Gale *et al.* (1991). Ruppert and Fox (1988: 100) have bestowed on *B. impressa* the "fanciful" common name "oyster mosquito." Another commercially important bivalve, *Mya arenaria* Linnaeus, 1758, is the host of *B. bisuturalis* in the Gulf of St. Lawrence, eastern Canada (Medcof 1948, as "*Odostomia*" *trifida*). *Crassostrea virginica* is the host of *B. bisuturalis* in New England (Loosanoff 1956, as *Menestho* Möller, 1842). The feeding behaviors of *B. bisuturalis* and *B. seminuda* are much like those of *B. impressa*. They were studied experimentally by Boss and Merrill (1965), Robertson and Mau-Lastovicka (1979), and Powell *et al.* (1987a). All three species of *Boonea* were found together on *Crassostrea virginica* at Assateague Island, Maryland, by Prezant *et al.* (2002: 343, 347). Merrill and Boss (1964) recorded the "reactions of hosts to proboscis penetration by" *B. seminuda*, and Bullock and Boss (1971) observed *B. bisuturalis* in the field north of Cape Cod, Massachusetts, with seven species of mollusks, not including *Crassostrea*.

Salinity tolerances and bathymetry

Most pyramidellids are fully marine, but some are estuarine. In southeast Asia a few species live in salinities as low as 0.5‰, and in the laboratory one was "easily kept in completely fresh water" (Brandt 1968: 277–279). *Boonea impressa* is the most euryhaline of the eastern American *Boonea* species, living in North Carolina in salinities down to 11‰, as determined experimentally by Wells (1961: 258). Low salinities caused by hurricanes eliminated some populations until

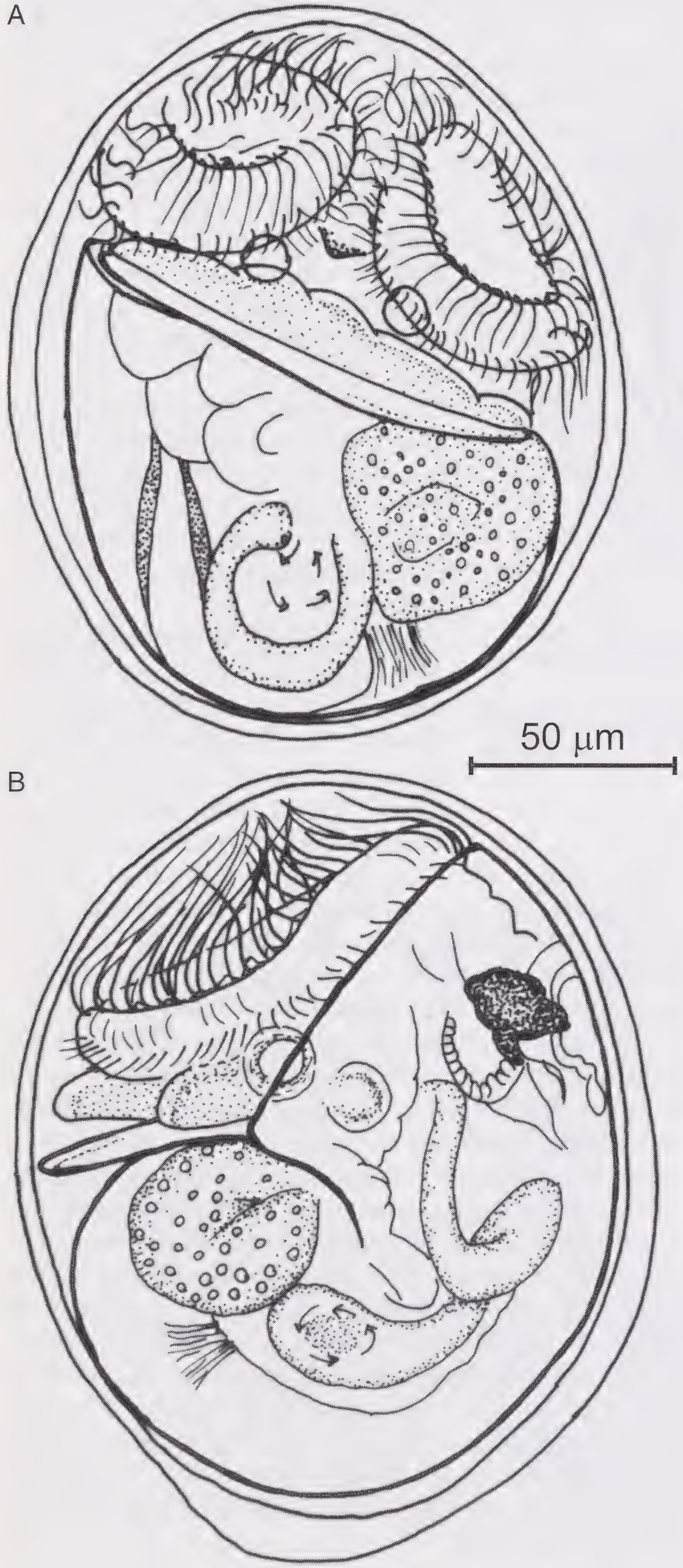

Figure 5. A–B, *Boonea bisuturalis.* Advanced intracapsular veliger in two views (**A** is oblique, and **B** lateral). Woods Hole. An operculum is present, but no pedal bristles.

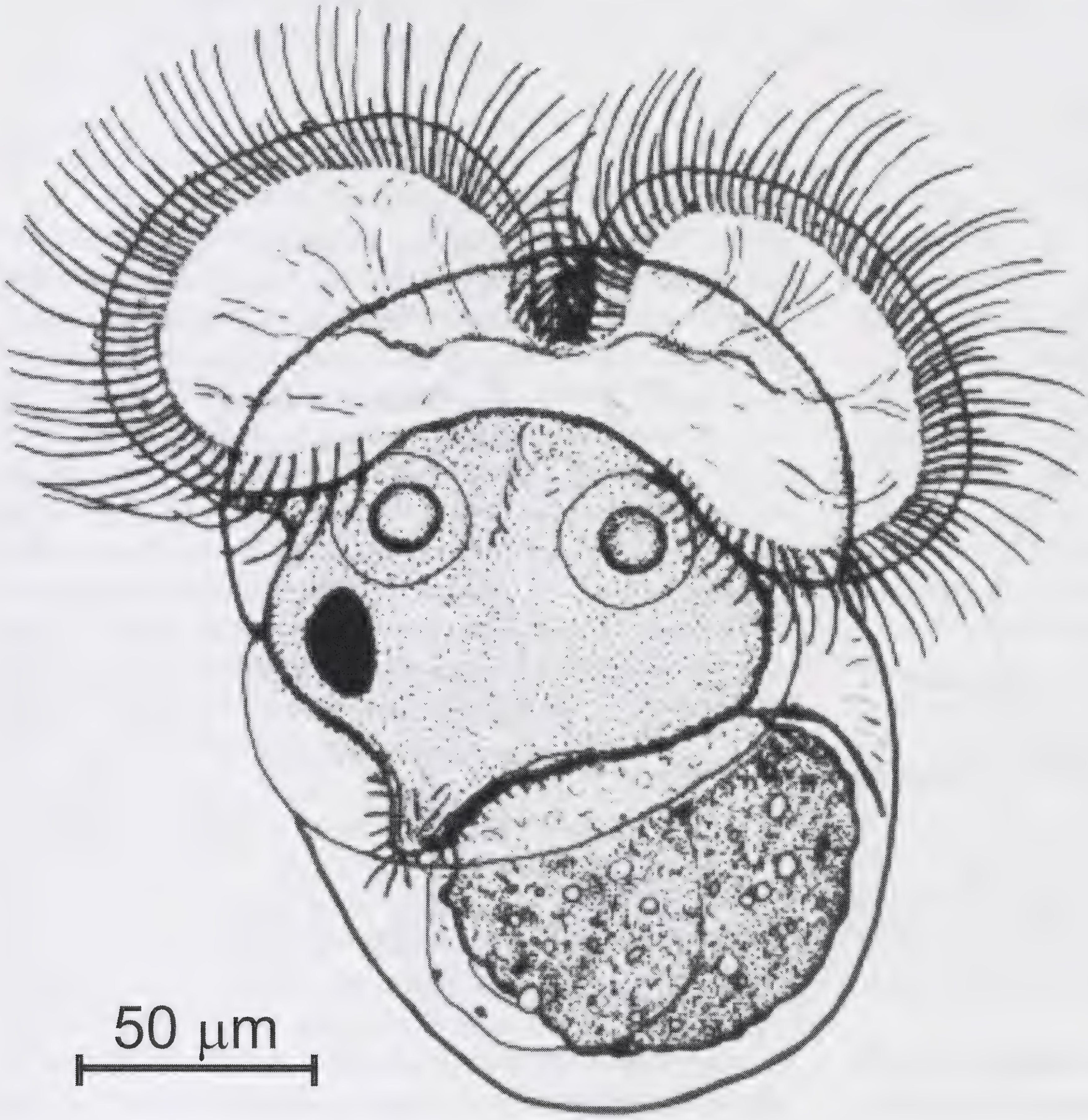

Figure 6. *Boonea bisuturalis.* Newly-hatched swimming planktotrophic veliger. Woods Hole. The conspicuous dark and opaque gland is the PMO. The paired statocysts are also conspicuous, but no eyes or tentacles are present at this stage. Pedal bristles are present.

they were recruited in the subsequent breeding season. The salinity range favorable for host *Crassostrea virginica* is wider: from 30‰ down to 5‰ (Galtsoff 1964: 404–407).

Cooper and Morse (1996: fig. 3) recorded salinities at Offatts Bayou, the precise locality of the studied population of *Boonea* "*impressa*" at Galveston, Texas: 22 to 23‰ in March, and 31 to 34‰ in September (at depths of 0 to 9 m). Parker (1959) gave data on salinities and temperatures where *B.* "*impressa*" lives in Rockport area bays, including Aransas Pass, Texas. *Boonea bisuturalis* has been reported in a salinity of 19‰ at the unusual depth (for an estuary) of 20 m in Chesapeake Bay (Merrill and Boss 1966). No salinity data for living *B. seminuda* appear to have been published, but Odé (1993: 54) mentioned that it is rare in coastal bays of Texas and lives mainly offshore. Bird (1970: 1655) evidently had only empty shells of the two species in North Carolina, but his data indicate that *B. impressa* occurred in much lower salinities than *B. seminuda. Boonea* "*impressa*" in Texas lives down at least to 20 m (Odé 1993: 54). Odé (1993: 55) recorded *B. seminuda* shells in Texas as deep as 101 m; living, it was only found down to 22 m. *Boonea* subtidal bathymetry is otherwise little known.

Temperatures

On the northeast United States seaboard there are major differences between summer and winter shallow sea temperatures. In winter, there is a gradient between these low temperatures in the north to higher temperatures farther south, with a disjunction at Cape Hatteras, North Carolina. This separates the Virginian Faunal Province from the more southerly and still warmer Carolinian Faunal Province (Coomans 1962). In summer, the Hatteras temperature barrier is somewhat minimized, and temperatures are similar all the way between Cape Cod (including Woods Hole), Massachusetts, and Cape Canaveral, Florida (Parr 1933; Wells and Gray 1962; Wells 1965). None of the three *Boonea* species studied here have range end points at Cape Hatteras.

The two *Boonea* species extending into New England and Canada are highly eurythermal. This is necessarily the case for species with such wide north-south ranges. Also, pockets of warm water occur in the north. Borei (1965) observed "*Odostomia trifida*" in a "warm lake" semi-isolated by a shallow sill from the open sea at Mount Desert Island, Maine, northern New England (specifically Upper Pretty Marsh "Pond"). This had a July and August temperature range of 16.0° to 23.2° C, near that at Woods Hole, the other side of Cape Cod, at the same time, but spawning was not looked for. The open sea temperature near there at the same time was 13° to 14.5° C.

Woods Hole dockside maximum and minimum weekly mean temperatures between 1965 and 1970 were -2° to +2° C in January and February, and 19.5° to 23.5° C in July and August (unpublished WHOI records). In North Carolina, south of Cape Hatteras, on the stretch of coast where Beaufort is located, water temperatures recorded along open beaches were 22° to over 25° C in late June to late July (Wells and Gray 1960). The shallow Newport River estuary adjacent to Beaufort has mean sea temperatures within 25° to 27° C in summer and as low as 12° C in winter (Williams 1966: 702,

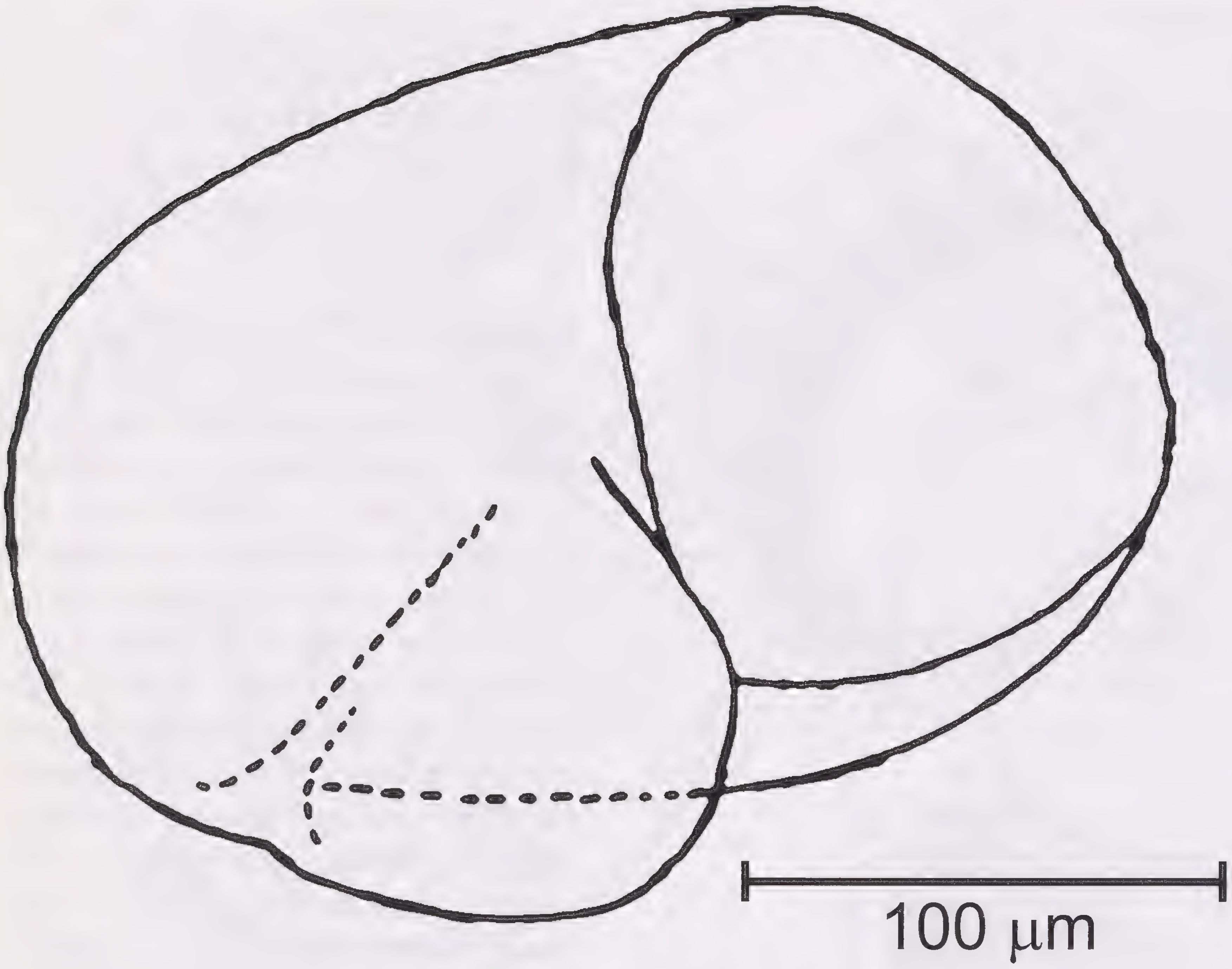

Figure 7. *Boonea bisuturalis.* Camera lucida outline drawing of a planktotrophic veliger shell. Woods Hole. The orientation is conventional, with the hyperstrophic aperture on the right. The dashed lines indicate features seen in transparency.

703). The maximum annual temperature range in the estuary headwaters was from 4° to 30° C. Sea temperatures at Offatts Bayou, the precise locality of the studied population of *Boonea* "*impressa*" at Galveston, Texas, were recorded by Cooper and Morse (1996: fig. 3): 14° to 16° C in March and 29° to 31° C in September (at depths of 0 to 9 m). Harry (1976: 138, 139) recorded nearby temperatures as low as 8° to 10° C in January and March. Tunnell (1973) recorded "*Odostomia*" *seminuda* quite abundant at a depth of 14 m on Seven and One-half Fathom Reef, 3.2 km offshore from Padre Island, Texas. Bottom temperatures there ranged from a low of 13.2° C in winter to a high of 30.1° C in late summer.

Table 2. Major reproductive features of *Boonea seminuda* at Woods Hole, Massachusetts and North Carolina. They both have B-type protoconchs. Ranges, means, and sample sizes (in parentheses).

Locality	Woods Hole	North Carolina
Eggs per egg mass	14–216 104.8 (45)	
Egg capsule lengths (µm)	137–165 150.9 (44)	
Egg diameters (µm)	65–78 67.9 (69)	65–69 67.3 (10)
Hatching shell diameters (µm)	146–158 150.8 (15)	141–151 146.5 (10)
Reproductive mode	Planktotrophy	Planktotrophy

Breeding seasons

Boonea spawning and spermatophore occurrence times were recorded in Robertson (1978). All appear to have approximately annual lifecycles. In Massachusetts *B. seminuda* and *B. bisuturalis* breed only in summer. *Boonea seminuda* egg masses were seen between early July and early October. *Boonea bisuturalis* egg masses were seen between early June to mid-September ("abnormal" eggs in late September). *Boonea bisuturalis* on the Delaware Bay coast of New Jersey had egg masses with hatching veligers in August. In North Carolina, *B. seminuda* breeds over a more "extended period", including even January (Wells and Wells 1961: 156; Wells *et al.* 1964: 577). An April shell length frequency distribution was wide and unimodal (their fig. 6). Wells (1959: 141) observed North Carolinian *B. impressa* spawn from early May to mid-September. He used shell length-frequency distributions to determine its annual lifecycle there. Hopkins (1956) indicated that *B. impressa* breeds in summer in South Carolina. In the Gulf of Mexico, *B.* "*impressa*" appears to breed all year but perhaps not in each population. Animals from northwest Florida spawned in February and March. I observed egg masses from Galveston, Texas, only in some non-summer months: late November to late December and from early March to early April. At Port Aransas, *B.* "*impressa*" reproduction and recruitment occurred all year but was "markedly higher in early summer (May–July)" according to White *et al.* (1985: 44).

Shell sizes

According to Rosenberg (2010), maximum attained shell lengths of *Boonea seminuda, B. bisuturalis* and *B. impressa* are, respectively, 6.9 mm, 5.8 mm and 6.7 mm. The largest *B. seminuda* that I have observed had a shell 7.0 mm long. I collected it alive at Northwest Gutter, Elizabeth Islands, near Woods Hole, on June 30, 1966. It is possible that this individual over-wintered twice, which would have been unusual. The lifecycle of *B. bisuturalis* at Woods Hole is closely similar. An early September shell length distribution, based on 138 animals, shows two cohorts. The most numerous one is modal at 1.2 to 1.6 mm, and the smaller one at 3.8 and 4.4 mm. The largest observed Galveston *B.* "*impressa*" shell was 6.4 mm long.

Rearing *Boonea bisuturalis* larvae

Using algal cultures and crude techniques, I succeeded in rearing a few *Boonea bisuturalis* from eggs to well past metamorphosis in the laboratory (Robertson 1967). This was the first planktotrophic pyramidellid or other parasitic gastropod to be so reared. Using the same techniques, I failed to rear *B. seminuda,* a less hardy species in the laboratory. Using more sophisticated techniques, Leibowitz (1979) succeeded in rearing numerous *B. bisuturalis.* It is beyond the scope of this paper to detail his or my methods, but the developmental stages are first illustrated here. Detailed methods for rearing planktotrophic larval *Aplysia* Linnaeus, 1767 through metamorphosis in the laboratory were given by Kriegstein *et al.* (1974). Leibowitz provided ideas for that study. Other methods for rearing "opisthobranch" larvae were reported by Harrigan and Alkon (1978).

Erroneous reports

As Wells (1959: 141) pointed out, the "egg capsules" of *Boonea impressa* portrayed by Perry and Schwengel (1955, pl. 46, fig. 322, a, b, as *Odostomia*) are not those of a pyramidellid. They match those of the nassariid *Nassarius vibex* (Say, 1822) illustrated by D'Asaro (1993: 175, fig. 15). The egg capsules supposed to be of *N. vibex* that were illustrated by Perry and Schwengel (1955, pl. 51, fig. 347) are actually those of the ocenebrine muricid *Urosalpinx perrugata* (Conrad, 1846) (D'Asaro, 1991: 50, fig. 35, 1993: 207, table 8).

MATERIALS AND METHODS

Populations sampled

Boonea bisuturalis and *B. seminuda*, the two species at Woods Hole, southern Massachusetts, were less than 1 m deep at low tide, and were collected in the late spring, throughout summer, and in the early autumn, beginning in 1966. *Boonea bisuturalis* was common at the shore adjacent to Flume Pond, south of Gunning Point, 4 km east-northeast of Woods Hole, on the eastern coast of Buzzards Bay, often parasitizing introduced *Littorina littorea* (Linnaeus, 1758) on rocks, which had been there only since about 1875 (Dexter 1961,Wells 1965) (*Crassostrea* was absent from that habitat.) *B. seminuda* also occurred there in low numbers, but the studied populations were parasitizing shallow subtidal *Crepidula fornicata* at the north end of Quissett Harbor, 3 km northeast of Woods Hole, and from Wreck Point, Cape Lookout, 16 km southeast of Beaufort, North Carolina (also on *Crepidula fornicata*; it also occurred there on *Argopecten irradians* (Lamarck, 1819)). Cursorily, in August, 1971, I studied *B. bisuturalis* from *Crassostrea virginica* near the mouth of Dias Creek, Pierce's Point, Cape May County, on the New Jersey coast of Delaware Bay.

Boonea impressa was studied in North Carolina when I was at the Duke University Marine Laboratory, Beaufort, in the summer of 1977. Even though this is a common species there and this is within the known breeding season (Wells 1959), I had difficulty finding adult populations and their egg masses. The few studied were parasitizing shallow subtidal *Crassostrea virginica* at the west side of Banks Channel, Wrightsville Beach, 14 km east-southeast of Wilmington, North Carolina. I also had living *B.* "*impressa*" sent to me by correspondents (listed in Acknowledgments) from Virginia, South Carolina, Georgia (Jekyll and Sapelo Islands), northwest Florida (Dixon Bay, Wakulla County), and Texas (off 61st St., Offatts Bayou, on the inner coast of Galveston Island, West Bay). Animals from all these localities were from *Crassostrea virginica* and laid eggs in the laboratory. Voucher specimens (98 shells) of *B.* "*impressa*" from Offatts Bayou, live-collected by Mrs. Boone in 1968 and dried, are at ANSP (no. 319083). Galveston is 273 km northeast of Port Aransas, where White *et al.* (1985) studied *B.* "*impressa*". All the newly studied populations probably were from fully marine or slightly brackish waters. I have not observed *Boonea* occurring in pairs and laying paired egg masses.

Laboratory maintenance, temperatures, and hardiness

At WHOI, mature animals were maintained in 15 cm diameter glass bowls in the laboratory. In summer, both laboratory running seawater and water in rearing dishes ranged from about 17° to 21° C. Water was changed every other day. Either in the presence or absence of their hosts, within three or four days after being collected many of the animals laid egg masses, either on the glass or on one another's or the hosts' shells. Detached masses were quickly infested with bacteria and protozoans, did not hatch, and were discarded. Developmental studies were therefore based on laboratory-laid eggs in still-attached masses. Uncleaved eggs were measured with an ocular micrometer in a compound microscope. Unless indicated otherwise, all drawings were done with the aid of a camera lucida or from photographs. *Boonea bisuturalis* is remarkably hardy: it survives for days in containers of seawater containing putrefying hosts. The cause of death seems to be its shell dissolution in the acidity.

Institutional abbreviations

ANSP = Academy of Natural Sciences of Philadelphia; MCZ = Museum of Comparative Zoology, Harvard University; USNM = National Museum of Natural History, Smithsonian Institution; WHOI = Woods Hole Oceanographic Institution, Massachusetts.

RESULTS

Protoconch morphologies and sizes

The three *Boonea* species uniformly have smooth, B-type protoconchs consisting of about 1½–1¼ tilted heterostrophic whorls. They have about the same size ranges. I measured the intact protoconch diameters on all the shells in the *Boonea bisuturalis* + *B. impressa* complex at ANSP, MCZ and USNM (grouped because they were not different). Those from Maine to northwest Florida ranged from 200 to 280 µm (mean 250 µm; *N* = 494). Those from Texas (all localities) ranged from 220 to 250 µm (mean 230 µm; *N* = 92). In Massachusetts, *B. seminuda* protoconchs are about 217 to 295 µm in diameter (Fig. 2 A, C); in Brazil they are well within the same size range: about 232 to 262 µm (Pimenta *et al.* 2009: figs. 13, 14). Porter *et al.* (1979, fig. 4) compared the protoconch widths of North Carolinian *B. seminuda* and *B. impressa*. The means were nearly the same (within 225 and 235 µm), but the variability they recorded seems unaccountably wide.

Egg masses (Fig. 3)

Those studied were all laid in the laboratory soon after collection of the adults. They were attached to the walls of glass containers or, occasionally, the shells of living conspecific or non-conspecific adults. They were gelatinous and formless like those of other pyramidellids. Single masses are up to about 4.4 mm wide. The egg capsules (inside the masses) are connected end to end by chalazae. Egg arrangement is not consistently linear as they commonly are in higher "opisthobranchs." No extra-capsular material or "yolk" was observed.

Capsule and egg numbers per mass (Tables 1–4)

These can be augmented by multiple or gregarious spawnings (as in *Fargoa:* Robertson 2012b). In *Boonea* species studied by me there was invariably one egg per capsule.

Egg capsule sizes and morphologies

Planktotrophic forms have smaller capsules than non-planktotrophic ones, paralleling egg sizes. In *Boonea seminuda,* the lengths (outer diameters) were 137 to 165 µm. In *B. bisuturalis* (Fig. 4) they initially were at least 162 µm. The capsules appear to swell slightly during development. In Fig. 5A, the capsule length is about 176 µm; in Fig. 5B it is about 192 µm. In Galveston *B.* "*impressa*" capsule lengths were 296 to 403 µm. *Boonea* capsules are all elliptical and about the same shape. Their mean length:width ratios varied between 1:0.77 and 1:0.91. Egg capsule length versus egg size ratios were about 1:0.32 (Fig. 4). Each capsule wall is uniformly thin, and 1-layered. There are surrounding layers of mucus (not previously reported in pyramidellids). Alternating empty and egg-bearing capsules such as those illustrated in Robertson (2012a, Fig. 5) were not observed.

Table 3. Major reproductive features of *Boonea bisuturalis* at Woods Hole, Massachusetts and Delaware Bay, New Jersey. They both have B-type protoconchs. Ranges, means, and sample sizes (in parentheses).

Locality	Woods Hole	Delaware Bay
Eggs per egg mass	21–276 97 (32)	
Egg capsule lengths (µm)	162–192 176.7 (72)	
Egg diameters (µm)	68–80 75.2 (65)	
Oviposition to hatching (days)	4	
Hatching shell diameters (µm)	158–180 170.7 (20)	160–174 168.7 (10)
Veligers planktonic (days in lab)	16–20	
Shell diam. at metamorphosis (µm)	about 250	
Reproductive mode	Planktotrophy	Planktotrophy

Egg sizes (Tables 1–4)

Eggs of planktotrophic *Boonea seminuda, B. bisuturalis,* and North Carolinian *B. impressa* are all 65 to about 80 µm in diameter. Those of *B.* "*impressa*" at Port Aransas, Texas, where there is lecithotrophy, are 182 to 238 µm in diameter

Table 4. Major reproductive features of *Boonea impressa* at North Carolina, and a (conspecific?) population at Galveston, Texas. They both have B-type protoconchs. Ranges, means, and sample sizes (in parentheses).

Locality	North Carolina	Galveston
Eggs per egg mass	15–40[1]	1–39 11.1 (28)
Egg capsule lengths (µm)		296–403 341 (39)
Egg diameters (µm)	about 80[2] (1)	212–236 mean 220
Oviposition to hatching (days)		20–28 mean 23.6 (7)
Hatching shell diameters (µm)	154–162 158.0 (10)	240–276 mean 257
Veligers planktonic (days in lab)	about 15–20	0
Reproductive mode	Planktotrophy[3]	Intracapsular metamorphosis

[1] Data from Wells (1959: 141)

[2] One early embryo

[3] Conclusion supported by Wells' (1959) data

(Table 1). Those of *B.* "*impressa*" at Galveston, Texas, where there is consistent intracapsular metamorphosis, are 212 to 236 μm in diameter. All eggs were pale cream-colored.

Intracapsular albumen (Fig. 4)

These granules are present in all three species (and perhaps all other pyramidellids). It disappears during development, as in sacoglossans (Clark *et al.* 1979) and nudibranchs, and may be important in providing extra-ovular food for the pre-hatching larvae. No intracapsular, extra-embryonic material or "yolk" was observed.

Winter shell growth cessation

The intertidal and shallow subtidal *Boonea* and *Fargoa* species at Woods Hole, like probably most organisms, undergo this cessation. In spring, the earliest-grown portions of the teleoconchs, grown late the previous year, have become opaque, chalky, etched or abraded, or "algal excoriated" (Miller 1983). The clearly demarcated newly grown teleoconch parts are translucent and unabraded. (Similar observations were made on a New England *Turbonilla* by Wharton 1976.) In summer at Woods Hole and in a refrigerator at a temperature below about 10° C, the two *Boonea* species did not move, feed, grow, or spawn. Galtsoff (1964: 407, 408, fig. 368) recorded parallels in the *Crassostrea virginica* host. He recorded annual mean monthly sea temperatures for Long Island Sound and northwest Florida. At both places there was *Crassostrea virginica* "hibernation" below 8° C and "reproduction" above 20° C.

Boonea seminuda (Fig. 3; Table 2)

The egg masses and capsules match those of *Boonea bisuturalis* in size and morphology. The eggs are slightly smaller, but the newly-hatched planktotrophic veligers and protoconchs appear identical. Durations of their pelagic larval stages presumably are similar. Only Woods Hole and North Carolinian populations were studied. *Boonea seminuda* was stated by Wise (1996: 459), wrongly citing me, to be lecithotrophic.

Boonea bisuturalis (Figs. 4–6; Table 3).

In *Boonea bisuturalis* the egg initially occupies a small part of the capsule's interior, which contains "albuminous" granules (Fig. 4). No polar bodies were observed. The embryos were initially of about the same sizes as the eggs, but became larger so that the capsules were almost filled by the young veligers before they hatched (Fig. 5A–B). The capsule wall is nearly even in thickness, and paired statocysts are conspicuous. So also is the dark and opaque PMO (Figs. 5B, 6). An operculum is present. At hatching, the larvae have no eyes or tentacles (Fig. 6). These appear in the late veliger stage. The unpigmented velum is fairly large and bilobed, with the right lobe slightly larger than the left. The digestive gland becomes green with chlorophyll if swimming larvae are fed *Isochrysis* in the laboratory. The early larva is already asymmetric, and late larval shells are distinctly hyperstrophic (Fig. 7) and shows water repellency. Pedal bristles were present. Fed veligers grew rapidly. A beating larval heart appeared late in development. Time from hatching to the pediveliger stage was 16 to 20 days in the laboratory and the shell diameter was then about 250 μm. The postlarva was about 385 μm long a week later when there was a change in coiling axis. Young snails began to parasitize hosts about 3 days later. Thus, the conversion from phytoplanktivore to benthic ectoparasite took only about 10 days. Young *Littorina littorea* were offered and were fed upon. (Bickell *et al.* 1981 studied thoroughly the morphogenesis of a nudibranch converting from a phytoplanktivore to a benthic carnivore). In one instance in the laboratory, a postlarva reared from the preceding summer with *L. littorea* as hosts attained a shell length of 4.5 mm by October 1. This size was about equal to the sizes of individuals in the field at that time.

Fragmentary data on *Boonea bisuturalis* from the New Jersey coast of Delaware Bay (Table 3) show that the sizes of newly-hatched veliger shells were 160 μm to 174 μm, *i. e.* close to the sizes of *B. bisuturalis* at Woods Hole.

Boonea impressa (Tables 1 and 4)

My evidence for planktotrophy in North Carolinian *Boonea impressa* may appear weak (Table 4). The single egg size (about 80 μm) was based on one early embryo, not a true egg. The data on hatching veliger shell diameters (154 to 162 μm) are more substantial. Also, Wells (1959, fig. 1 insert) illustrated an intracapsular late embryo with a somewhat smaller shell, about 137 μm in diameter, further evidence for planktotrophy there. Because *B. impressa* is abundant on the shallow North Carolina coast it is likely that the onshore transport of a pelagic larval "*Odostomia* sp." reported there by Shanks *et al.* (2000) was based on this species. If this inference is right, this is further indication of North Carolinian planktotrophy.

Fragmentary data (more egg diameters estimated from early developing embryo sizes, intracapsular development times, and more hatching sizes) suggest that *Boonea impressa* in South Carolina, Georgia, and the geographically disjunct population in northwest Florida are also planktotrophic. These data are not included in Table 4.

In striking contrast, the data in Table 4 show that *Boonea* "*impressa*" at Galveston, Texas, definitely has consistent intracapsular metamorphosis. Galveston egg masses were of about the same sizes and general appearance as those of *B. bisuturalis*, but the eggs and capsules were markedly fewer (1 to 39 per mass) and larger (capsules 296 to 403 μm long, and eggs 212 to 236 μm in diameter). Intracapsular development lasted 20 to 28 days, the whole veliger stage was passed inside the capsule, and the young all emerged crawling. Laboratory-reared specimens hatched at about the same size and with about the same morphology as *B. bisuturalis*. Hatchlings had about 1½ shell whorls and eyes and tentacles.

Late intracapsular Galveston *Boonea* "*impressa*" veligers

Velar lobes were present, ciliated, and not reduced in size. An operculum and a bright yellow and brown PMO were also present (also a dark purple spot posteriad). Pedal bristles were present. There were no eyes or tentacles. Ciliation in the esophagus and stomach was reduced, and the large digestive gland was full of yolk. Thus, the change from filter- to yolk-feeding is visible internally.

Data on North Carolinian *Boonea impressa* questioned

Wells *et al.*'s (1985) data on North Carolinian *Boonea impressa* are questioned here. They thought that *B. impressa* is lecithotrophic both in North Carolina and Port Aransas, Texas. In their abstract, they stated explicitly that reproduction and larval life history were not studied in North Carolina. Nevertheless, they presented data (their table 2) on the sizes of 24 supposed North Carolinian eggs from two egg masses: 190 µm to 222 µm (mean 199 µm). This accords with their and my Texan data (their data are not greatly different), but emphatically not with my North Carolina data. These show planktotrophy (Results and Table 4). Both planktotrophy and lecithotrophy in North Carolina seem unlikely, although a few lecithotrophic nudibranch species have been shown to be facultatively planktotrophic (Kempf and Hadfield 1985, Kempf and Todd 1989, Krug 1998). I suggest that White *et al.* mistakenly thought that some of their Port Aransas eggs were from North Carolina.

Larval development from oviposition to hatching in the laboratory at Port Aransas was recorded by White *et al.* (1985) as requiring only 3.3 to 4.8 days. This contrasts drastically with the Galveston data of 20 to 28 days in the laboratory. Their reported durations agree with the data for planktotrophic *Boonea bisuturalis* in the laboratory at Woods Hole: about four days. These are the shortest known pre-hatching development times in pyramidellids, yet they reported lecithotrophy. I suggest that somehow they made a labeling mistake. They recorded a non-feeding planktonic stage lasting about one week. Did they mean the time between hatching and metamorphosis? Judging from their fig. 3B, the hatching shell diameter was about 265 µm.

White *et al.* (1985: table 2) measured 54 eggs from five Port Aransas egg masses. The size range was 182 µm to 238 µm (mean = 206 µm), i.e. there was high variability. This agrees fairly well with the Galveston data (212 µm to 236 µm; mean 220 µm) (Table 4). White *et al.* mentioned that eggs in a single egg mass tended to be of similar sizes. One of their egg masses contained the smaller eggs, while the four others all contained the larger ones. Perhaps larger individual adults lay the slightly larger eggs? According to White *et al.* (1985) 20 to 250 eggs were laid per Port Aransas egg mass. Considering the large egg sizes and the comparable numbers in Galveston (1 to 39; Table 4) and in other pyramidellids, the much higher number must have been based on several or many congealed masses that may have been spawned gregariously (compare *Fargoa bartschi* (Winkley, 1909): Robertson 2012b).

DISCUSSION

It is now clear that B- and C-type protoconchs do not generally reflect non-planktotrophy. *Boonea* species or populations, all with B-type protoconchs, can be planktotrophic, lecithotrophic, or have intracapsular metamorphosis. There appears as yet to be no evidence that C-type protoconchs ever reflect "direct" development (Robertson 2012b). Non-planktotrophy has systematic implications because at least theoretically it restricts larval dispersal.

The only other "opisthobranch" species known to me to have all three modes of larval development is the plakobranchid sacoglossan *Elysia cauze* Marcus, 1957 (Clark *et al.* 1979). Unlike *Boonea* "*impressa*" this occurs in one population, the variation is seasonal, and this is apparently caused by variable use of extra-capsular yolk (unknown in pyramidellids).

B-type protoconchs in other pyramidellid genera have wrongly been thought to reflect modes of larval development. For example, some living as deep as 2500 m are known, or have been inferred, to undergo vertical migrations and be planktotrophic in the photic zone (Bouchet and Warén, 1994: table 5.1). A B-type protoconch of *Turbonilla hamonvillei* Dautzenberg and Fischer, 1896 was illustrated by Bouchet and Warén (1979: fig. 12), and it was from a depth of 1950 to 2100 m. A B-type protoconch does not now prove that it was planktotrophic.

This paper reports apparently the first known exception to Thorson's (1950) "shell apex theory." It is not always possible to determine the mode of development from protoconch characters alone, as was indicated by Thiriot-Quiévreux (1980: 72). Protoconch shape is not necessarily the "result of developmental mode" (Wise 1996: 443). Hadfield and Strathmann (1990) reported that egg size and size or shape of the protoconch in four lecithotrophic trochoideans do not reflect whether the larvae are benthic or pelagic. Vetigastropods such as these, which are not known ever to be planktotrophic, never have multispiral protoconchs in the sense of Thorson. Thus, they did not contradict the theory. Despite the uniformly B-type protoconch morphology of *Boonea impressa* and the uniform size ranges of these, this species varies in mode of reproduction from place to place. In North Carolina it is planktotrophic, at Port Aransas, Texas, it is lecithotrophic, and at Galveston, Texas, there is intracapsular metamorphosis. It is unknown whether other *Boonea* species vary in this way, but there is much variation in the genus (see earlier under *Boonea* protoconchs). Pimenta *et al.* (2009: 49) were prescient in stating that the "larval ecology of pyramidellids cannot be determined from shell characters alone." As even Thorson's (1946) data showed, pyramidellid protoconchs sometimes reflect phylogeny more than they do larval ecology. It may be that his theory applies to prosobranchs but not all "opisthobranchs."

In the *Boonea bisuturalis* + *B.* "*impressa*" complex the most profound teleoconch disjunction occurs between Long Island Sound and Chesapeake Bay, while the greatest reproductive disjunction occurs between North Carolina and somewhere in the northeastern or northern Gulf of Mexico. They may be two species or both may be one widely dispersed and highly and continuously variable biological species. Alternatively, there may be allopatric poecilogony (as defined in Hoagland and Robertson 1988, and Bouchet 1989). (The term poecilogony is frequently misused; for example, it was wrongly defined in Lincoln *et al.* 1998.) For true poecilogony there would need to be two or more distinct intraspecific reproductive modes. The term does not apply to cases in which there is wide but continuous variability, as could be the case in *B.* "*impressa*". Poecilogony is not just variable intraspecific larval development. The difficulty of distinguishing poecilogony from sibling species was well shown by Miles and Clark (2002). If there is a geographical continuum of different reproductive modes, and if only two discrete populations are compared, a likely wrong conclusion is that there is poecilogony, as indicated by Hadfield and Strathmann (1996). According to Rasmussen (1951) there is a salinity-related allopatric continuum in Baltic *Brachystomia* "*scalaris*" (Macgillivray, 1843). Differing extrinsic factors such as weather, degree of wave action, or adult food supply may have induced some of the recently reported cases of "opisthobranch poecilogony", for example that in *Alderia* Allman, 1846 species (Sacoglossa) reported by Krug (1998) and Krug *et al.* (2007). More closely spaced population sampling and laboratory experiments could shed light on both these cases. Reproduction at Port Aransas and Galveston is not greatly different because "*Turbonilla*" *cummingi* Hori and Okutani, 1997 is known to have sympatric lecithotrophy straddling intracapsular metamorphosis. The same appears to be true of at least the Danish populations of *Brachystomia* "*scalaris*" except that the variation there is micro-allopatric.

To be noted is that the most northern *Boonea* species are planktotrophic, while the southernmost populations known (in Texas) are non-planktotrophic. This disagrees with another of Thorson's (1950) "rules" (Clark and Goetzfried 1978: 287).

Another possibility is that at least the Galveston population is a chemically-induced ecotype. Offatts Bayou, the precise location of the intracapsular metamorphic population studied here, has unusual water chemistry (Gunter 1942, Cooper and Morse 1996): the estuarine water is oxidic in winter and highly sulfidic in summer, especially at a depth below 4 m. *Boonea* "*impressa*" was collected in water shallower than this, but could be affected when surface waters become mixed with the deeper sulfidic layer. Sulfide toxicity was discussed by Van Dover (2000: 183, 197–200). Toxic metal traces were associated with the sulfides in the Galveston bottom sediments (Morse *et al.* 1993 [cited by Cooper and Morse 1996]). The pollution is anthropogenic, and already Eckhardt (1968) was writing about the "death of Galveston Bay" and Carter (1970) was calling it "an estuary in crisis." This was before Mrs. Boone collected living *B.* "*impressa*" for me at Offatts Bayou (in 1977 and 1978). Sediments at some *Boonea* habitats on the eastern American seaboard, at bay side Assateague Island, Maryland, had hydrogen sulfide odors (Prezant *et al.* 2002), but these were not as extreme. Conditions at Port Aransas are unknown to me. Differing salinities might also affect egg sizes and modes of development, as in perhaps the case of at least Danish *Brachystomia* "*scalaris*". I have no data bearing on this in *Boonea* "*impressa*."

If the differences are genetic a final possibility, and the most probable one, is that *Boonea* "*impressa*" is one or several different, unnamed, and allopatric sibling species on the Texas or other Gulf of Mexico coasts. White *et al.* (1985) compared adult North Carolinian and Texan *B.* "*impressa*" shells. These showed high variability, but they also found that "adult snail size, whorl length, whorl width, and the number of spiral cords [differ] significantly". The differences seem minor to me. Mensural components of the feeding apparatus appeared the same. Robertson (1978) showed that Texan spermatophores are consistently larger than those from northwest Florida and North Carolina, yet in Texas the shells attain slightly smaller sizes (6.4 versus 6.7 mm). Tunnell *et al.* (2010: 42–43, 261, fig. 3.17, figs. 3–4) recorded "narrower" and "stout" forms of *B.* "*impressa*" within Texas, but these seem to be within the range of intraspecific shell variation. In recent years more and more sibling species have been discovered, but not yet definitely in pyramidellids (but recall Høisæter 1989, on *Brachystomia* "*rissoides*" (Hanley, 1844)). For descriptions of new species to be justified there would need to be comparative micro-anatomical and molecular studies. Schander *et al.* (2003) is the first intrafamilial molecular study of pyramidellids.

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C-type protoconchs and planktotrophy in small eastern North American *Fargoa* (Pyramidellidae)

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Abstract: C-type protoconchs have been thought always to reflect a "direct" or short pelagic development, an idea deriving from Thorson's (1946) study of the early development (besides planktonic veligers) of only one pyramidellid species. The small-shelled genus *Fargoa* Bartsch (1955), some ectoparasitic on serpulid polychaetes such as *Hydroides*, apparently uniformly has C-type protoconchs. The eggs and early larval development of *Fargoa dianthophila* (Wells and Wells, 1961) (shells < 1.8 mm) and *F. bartschi* (Winkley, 1909) (< 4.0 mm) were studied in the laboratory at Woods Hole, Massachusetts. The eggs were only 53 to 57 µm and 55 to 60 µm in diameter, respectively, and as would be expected from eggs this size both species are definitely planktotrophic. Few other pyramidellids or other "opisthobranchs" lay eggs this small, and the single *Brachystomia* (Monterosato, 1884) species studied by Thorson (1946) was also planktotrophic. The protoconch diameters of *F. dianthophila* and *F. bartschi* are, respectively, about 260 to 315 µm and about 210 to 230 µm, *i.e.*, surprisingly large. This variation parallels neither the nearly uniform egg size nor the disparate adult shell sizes. No attempt was made to rear the larvae in the laboratory because there were only a few newly hatched veligers and these protoconchs indicated much growth in the plankton. These *Fargoa* veligers may remain planktonic for longer times than planktotrophic *Boonea* Robertson (1978). One protoconch, an egg capsule containing a late blastula, and a newly hatched swimming veliger are illustrated. Small pyramidellids appear to lay the smallest eggs, but this seems not to be true of other "opisthobranchs."

Key words: small shells and eggs, long planktonic stages

C-type protoconchs, which have been described and illustrated in Robertson (2012a, Fig. 1C copied from Peñas *et al.* 1996), were thought by Thorson (1946) to reflect a "direct" or very short pelagic veliger stage. This idea was based mainly on protoconchs of eight Danish species, only one of which he studied alive: the higher salinity form of *Brachystomia* "*scalaris*" (Macgillivray, 1843), which he called *B. rissoides* (Hanley, 1844) (Fig. 1). Rodríguez Babío and Thiriot-Quiévreux (1974, 1975) followed Thorson (1946) in thinking that the C-type protoconchs of 15 species reflected "direct" or short pelagic stages. They did not study any of the living animals.

The only other previous data on the early development of C-type species are those of Lebour (1932: 118–119, pl. 1, figs. 8–16) on *Brachystomia* [*Odostomia*] *eulimoides* (Hanley, 1844) and Lebour (1936: 556, pl. 2, figs. 11–15) on "*Chrysallida*" [Carpenter, 1856] *decussata* (Montagu, 1803). Judging by her fragmentary data, the former species is lecithotrophic but the latter may show some growth in the plankton (it may undergo mid-water metamorphosis).

Two species of *Fargoa* Bartsch (1955: 80) were studied in the laboratory at Woods Hole, southern Massachusetts, U.S.A.: *Fargoa dianthophila* (Wells and Wells, 1961) and *F. bartschi* (Winkley, 1909). Fig. 2 shows one of their C-type protoconchs. Also following Thorson (1946), Wells and Wells (1961: 156) surmised that in the first of these a planktonic stage is suppressed. They too did not study its egg or larval development, and described the protoconch as "deeply immersed" and "oblique" (= tilted? which it is not). Wells and Wells (1961) did illustrate the differences between *Fargoa* and B-type *Boonea* Robertson (1978) protoconchs (respectively, figs. 3 and 5, and 7, all as "*Odostomia*" Fleming, 1813).

Robertson and Orr (1961) reported a C-type protoconch in "*Odostomia*" *chitonicola* E.A. Smith, 1899 and followed Thorson in thinking, probably without justification, that its larvae are non-planktonic. Young postlarvae were clustered with adults on the chiton host.

As first realized by Thorson (1952: 282, fig. 5), egg sizes at least in prosobranchs and bivalves are excellent predictors of modes of larval development. So they are too in "opisthobranchs" (Hadfield and Miller 1987). A 55 µm egg is among the smallest known in pyramidellids or nearly all other "opisthobranchs" and is a strong indication of full planktotrophy. They recorded only 3 non-planktotrophic "opisthobranch" species with < 95 µm eggs. Pyramidellid eggs up to about 90 µm are now known always to be planktotrophic. The present paper shows that the two studied *Fargoa* species have 53 to 60 µm eggs that are as expected planktotrophic.

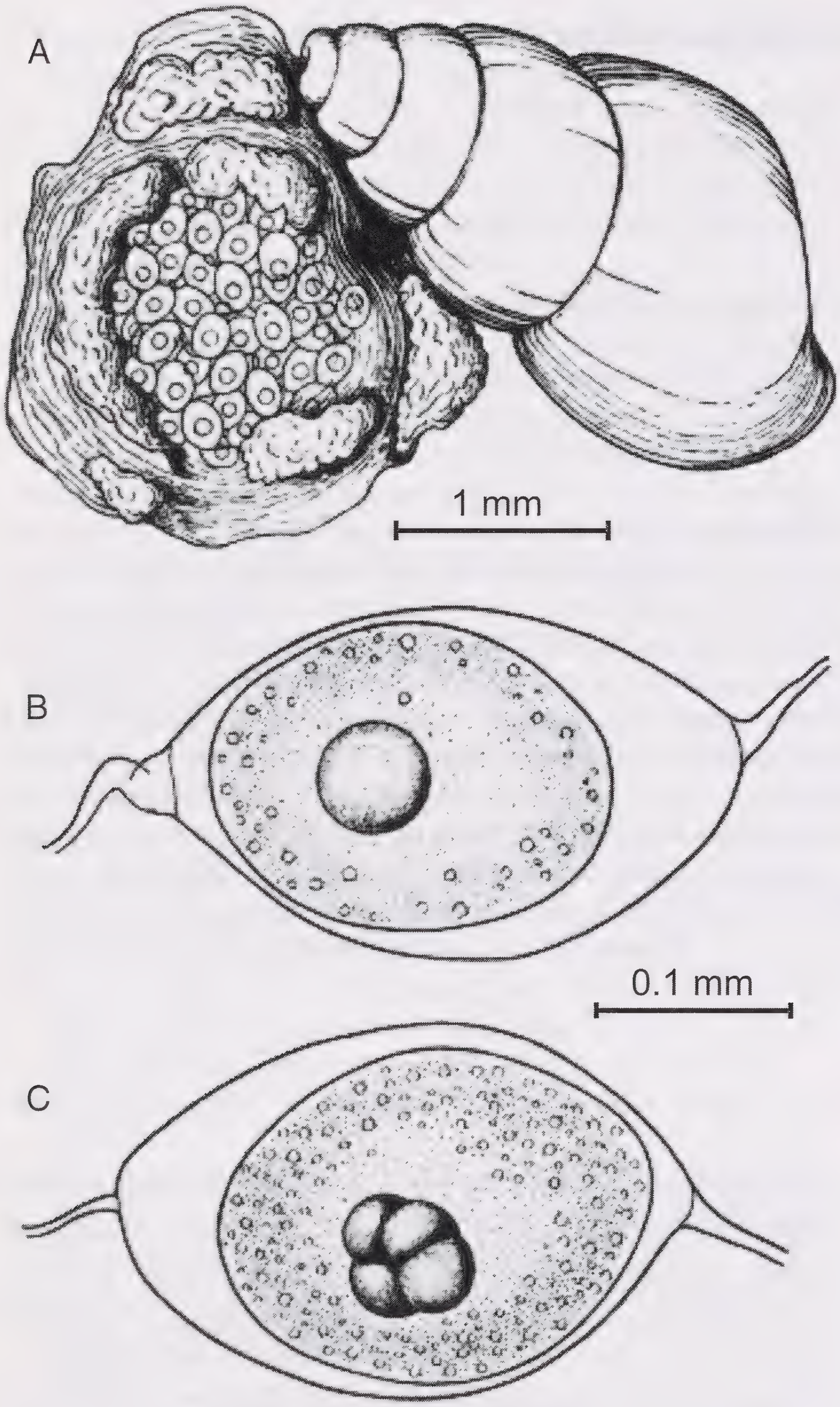

Figure 1. *Brachystomia* "*scalaris*" (higher salinity form). Southwest Sweden. Eggs and an early embryo, the only living pyramidellid at these early developmental stages studied by Thorson (1946: fig. 120 A–C, as *B.* "*rissoides*"). The species has a C-type protoconch which he believed to indicate non-planktotrophy. However, its 55 to 60 µm egg now proves planktotrophy. Note that the capsule wall is much thicker at one end than the other (as in *Fargoa* but not *Boonea*).

The systematics and zoogeography of *Fargoa* and the two species newly studied here were treated by Robertson (1978, 1996), Pimenta *et al.* (2009), and Rosenberg (2010). Except for Robertson (1978, 1996) and Harry (1984: figs. 13, 14, as *Fargoa* "*diathophila*"), adult *Fargoa* anatomy is unknown. *Fargoa* may well be a synonym of some earlier-named taxon known only from shells, of which there are many candidates (Schander *et al.* 1999). The genus has shell-attached spermatophores. At least the two species are host-specific ectoparasites on serpulid polychaetes in the genera *Hydroides* and less commonly *Eupomatus* (Robertson and Mau-Lastovicka 1979, Robertson 2006). It is unknown whether this is so of the whole genus. The two newly studied species both range at least from New England to Texas (neither is known from Brazil: Pimenta *et al.* 2009). They are sympatric to the extent that they occasionally occur together on the same individual worm. Like *Boonea,* they have disjunct ranges because of tropical, southern peninsular Florida (Robertson 2012b). They occur as far south on the east coast as the Indian River Lagoon (Mikkelsen *et al.* 1995: 114). The type locality of *Fargoa dianthophila* is Beaufort, North Carolina, and of *F. bartschi* Woods Hole, Massachusetts (Winkley 1909). The teleoconch sculptures of the two species are radically different (Robertson 1978: figs. 6, 8). As evidenced by numerous morphological characters, the two species are closely related (Robertson 1996). Adult *F. dianthophila* shells were illustrated originally by Wells and Wells (1961: figs. 1–3) from North Carolina. Texan *F. dianthophila* shells were illustrated by Odé (1971), Harry (1984: fig. 4) and Tunnell *et al.* (2010: 265). Adult *F. bartschi* shell variation in Massachusetts was illustrated by Robertson (1996: figs. 1–6).

Fargoa is about as euryhaline as *Boonea*. Adult *Fargoa dianthophila* in North Carolina was reported living naturally in a lasting salinity down to 15 ‰ by Wells and Wells (1961). They were correct in believing that its protoconch morphology is not salinity-induced. In the entire Indian River Lagoon, eastern Florida, where both *Fargoa* species were later reported, salinities generally ranged from 18 to 36 ‰, although extremes of 8 and 42 ‰ were recorded (Mikkelsen and Mikkelsen 1984: 165). In Texas, *F. dianthophila* was also plentiful in a "low salinity bay" but also "where salinities are much higher" (Odé and Speers 1972: 10–11).

The two species are subjected to the same wide temperature constraints as *Boonea* (Robertson 2012b). Reed and Mikkelsen (1987: 123) recorded *Fargoa dianthophila* from depths of 6 m, 42 m, and 80 m off the central eastern coast of Florida, near Fort Pierce (its southeastern-most known locality). They also published (pp. 109, fig. 6) detailed monthly and annual temperature data for these stations. At a depth of 6 m, the lowest temperature recorded (14° C) was in January, and the highest (31° C) in July. At greater depths, seasonality was obscured. At 42 m, the lowest and highest temperatures (8° C and 26° C) were both in July. At 80 m, the lowest temperature (7° C) was in July, and the highest (23° C) in May. *Fargoa bartschi* was first recorded from Texas by Odé and Speers (1972: 12, 14, 15, as *Eulimastoma* Bartsch, 1916). The greatest depth reported for its empty shells anywhere is 60 to 100 m, in calcareous rubble in salt domes off Louisiana (García and Lee 2004: fig. 71).

Fargoa dianthophila is small (< 1.8 mm), but is not the smallest pyramidellid species. There are at least six smaller described world species that are presumably mature at a shorter

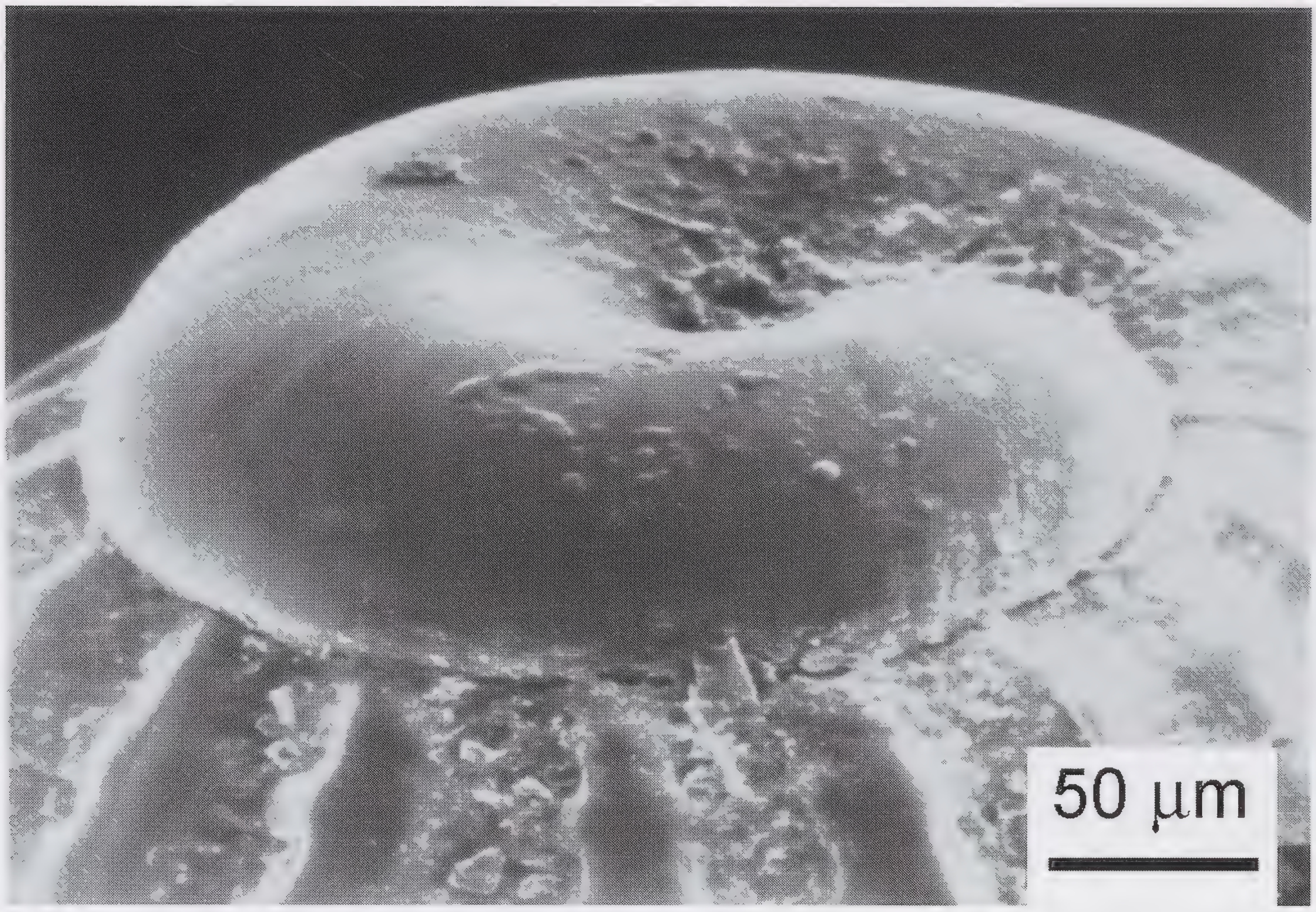

Figure 2. *Fargoa dianthophila.* Oblique lateral view of its C-type protoconch. Note the protoconch-teleoconch demarcation near the right margin. Woods Hole. Scanning electron micrograph.

shell length, all 1.1 mm. These are: "*Odostomia*" *mammillata* Carpenter, 1857 (Dall and Bartsch 1909: 233, pl. 30, fig. 8); *Pyrgulina nana* Hornung and Mermod (1923: 300, fig. 15); "*Odostomia*" (*Oda*) *edentata* Saurin (1959: pl. 2, fig. 6); "*Odostomia*" (*Siogamaia*) *transiens* Saurin (1959: pl. 3, fig. 11); "*Chrysallida*" *brattstroemi* Warén (1991: 100, figs. 32 A–C), and "*Chrysallida*" *dimidiata* Schander (1994: 17–18, fig. 1 d). A few slightly smaller described "species" may be juveniles. The smallest adult shell length that is functionally possible in the family may be about 1.1 mm.

Fargoa dianthophila is among the smallest "opisthobranchs" to have had its reproduction studied. Undoubtedly the smallest "opisthobranch" to have been so studied is the bivalved sacoglossan *Berthelinia australis* (Burn, 1960). Its shell length was "less than 1.0 mm", its egg was 54 µm in diameter, and it was studied by Wisely (1963). According to Burn (1960) the length of the largest animal was 5.5 mm, and of the largest shell 4.4 mm. This species may, like *Fargoa* species, be able sometimes to mature sexually at extra small sizes.

Fargoa bartschi is the largest known *Fargoa* species, rarely attaining a length of 4.0 mm in the north (not 4.4 mm as stated in Robertson 1996). North Carolinian shells are up to only 2.0 mm long, and correspondingly smaller spermatophores were observed on shells 1.0 and 1.3 mm long (Robertson 1978). The recipients may have been as fully mature as the donors, but some protandry could have been involved (Fretter and Graham 1949: 515).

The type species of *Fargoa* is *Fargoa calesi* Bartsch (1955: 80–81, pl. 16, fig. 2), a Pliocene fossil from Florida, and a synonym of Recent *F. bushiana* (Bartsch, 1909: 99, 100, pl. 13, fig. 44). The teleoconch attains a length of 3.1 or 3.7 mm. As yet, its hosts, eggs and larvae have not been studied, but Robertson (1978: 375, 376, fig. 71) studied its spermatophore. This is partly cuticularized and most closely resembles that of *F. bartschi.* One was attached to a shell only 1.4 mm long. Thus just as in *F. bartschi*, sexual maturity in *F. bushiana* can occur at extra small shell sizes. Odé (1968b) and others illustrated Texan shells. "*Chrysallida*" *buijsei* Jong and Coomans (1988: 123, pl. 19, figs. 646a, b), from the southern Netherlands Antilles, was synonymized with *F. bushiana* by Pimenta *et al.* (2009: 55–57, figs. 44–47), who confirmed its presence in Brazil. They illustrated two of its C-type protoconchs with low magnification scanning electron micrographs. *Fargoa bushiana* has a long North-South range but is virtually absent from the tropics in the middle of its range.

Fargoa gibbosa (Bush, 1909: 482) is a fourth definite *Fargoa* (Robertson 1978: 379). Bartsch (1909: 108, pl. 13, fig.50) illustrated it as *Odostomia modesta* "(Stimpson)" [: 1851]. Remarkably, *F. gibbosa* was stated by Odé (1968a: 17) to be "our most common [Texan] *Odostomia* [it] was present in countless numbers" (more so than *Boonea* "*impressa*" (Say, 1822)?). It attains a shell length of 3.5 mm, but its hosts, spermatophores, eggs, larvae and protoconchs are all unstudied. These two additional species document further the teleoconch sculptural diversity within the genus, another example of evolutionary teleoconch divergence in pyramidellids. *Fargoa gaudens* "Corgan" Odé (1993, 29–30, 32, text fig.) is juvenile and inadequately described and illustrated. The non-conchologically defined genus *Fargoa* appears to be restricted to the western Atlantic but may well be more widespread.

Both studied *Fargoa* species in southern Massachusetts and North Carolina have summer breeding seasons. Spawning and spermatophore occurrence times were recorded in Robertson (1978). *Fargoa dianthophila* egg masses were seen at Woods Hole in mid- and late July and mid-August. *Fargoa bartschi* egg masses were seen there in mid-July to late August. In North Carolina, *F. dianthophila* probably spawns in late May to July (Wells and Wells 1961: 156). Egg masses of North Carolinian *F. bartschi* were seen by me in late May and mid-August but could not be studied.

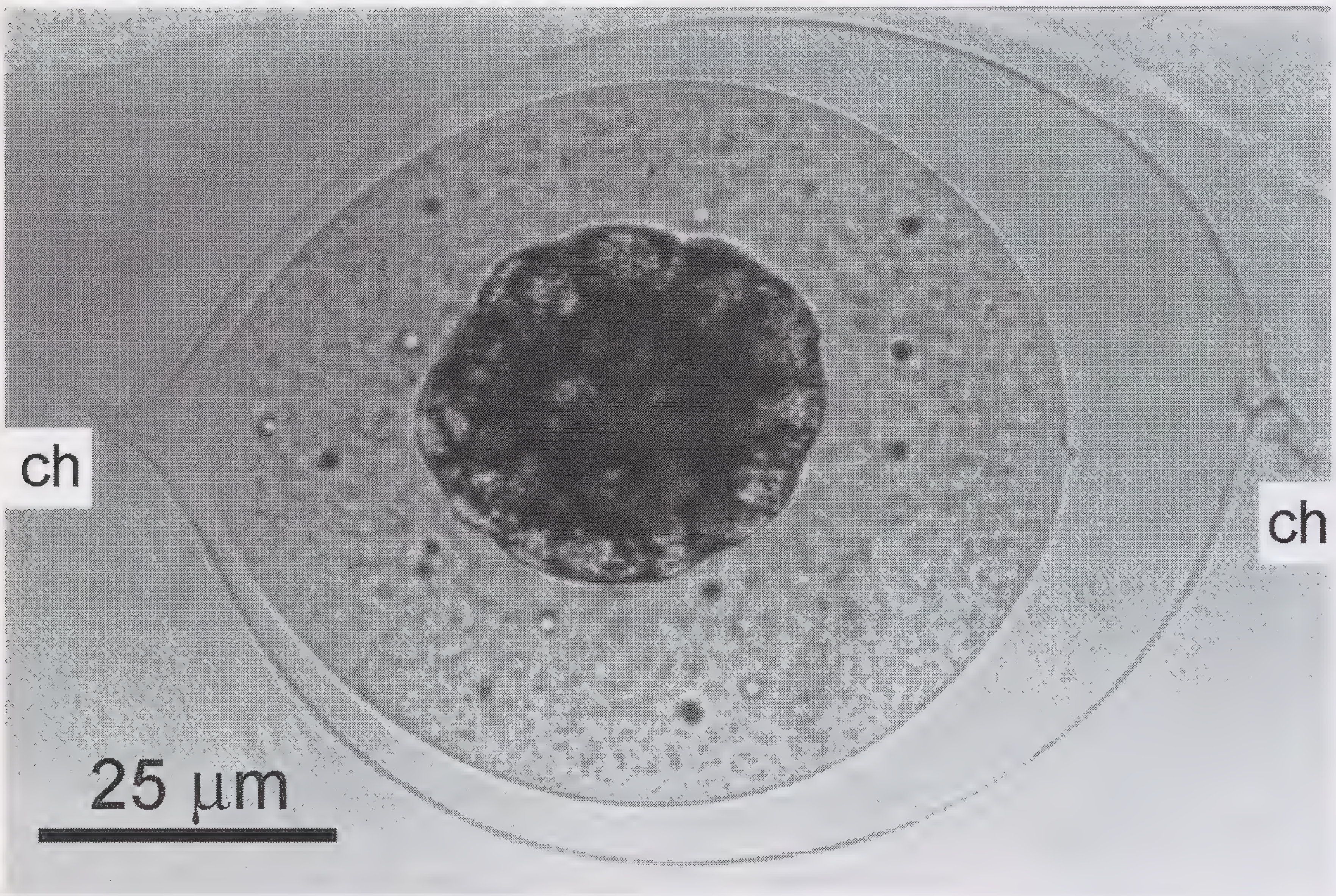

Figure 3. *Fargoa bartschi.* A late blastula in its egg capsule, surrounded by mucous layers and with intracapsular albuminous granules. Note that the capsule wall is much thicker at one end than the other. Woods Hole. Abbreviation: **ch**, chalazae.

Other information pertaining also to *Boonea* and other pyramidellids reported by Robertson (2012a, 2012b) is mostly not repeated here. Preliminaries to this paper were published in Robertson (1986, 1996).

MATERIALS AND METHODS

The two most studied *Fargoa* species were sampled together at the shore adjacent to Flume Pond, south of Gunning Point, 4 km east-northeast of Woods Hole, eastern Buzzards Bay, southern Massachusetts. They were 0 to 1 m deep at low tide, and were nearly always associated with the serpulid *Hydroides hexagonus* (Bosc, 1801) [*H. dianthus* (Verrill, 1873) is a synonym] under loose rocks. They were considerably rarer than *Boonea bisuturalis* (Say, 1822) at this locality, and were obtained during repeated field trips in the late spring, throughout summer, and in the early autumn, beginning in 1966. They were in fully marine salinities. In summer at Woods Hole, both laboratory running seawater and water in rearing dishes ranged from about 17° to 21° C.

RESULTS (Table 1)

Protoconchs and their sizes

The two *Fargoa* species both have C-type protoconchs consisting of about one whorl (Fig. 2). They are nearly isostrophic, not heterostrophic, and are not detectably tilted. Their sizes are different. Those of *F. dianthophila* were about 260 to 315 µm in diameter (Fig. 2) ($N = 3$), a size slightly larger than the largest metamorphosing *Boonea* developing from a much larger egg (Robertson 2012b). Those of *F. bartschi,* much the larger species, were smaller: about 210 to 230 µm in diameter ($N = 5$). These measurements were made on scarce Woods Hole specimens that were little abraded.

Egg masses

Egg masses of *Fargoa dianthophila* were found in the field on its host *Hydroides* "opercula" and on the outsides of their tubes near the openings. Egg masses of this species and of *F. bartschi* were also laid in the laboratory.

Egg numbers per mass

In *Fargoa dianthophila* there were 29 to 38 eggs per mass, and in *F. bartschi* 15 to 246. This upper limit undoubtedly reflected several or many congealed, perhaps gregariously spawned masses. The capsules observed invariably contained one egg (Robertson 2012b).

Egg capsule sizes and morphologies (Fig. 3)

In *F. dianthophila*, the outer egg capsule lengths were 137 to 158 µm. In *F. bartschi* they were 165 to 193 µm. Their mean length:width ratios were, respectively, 1:072 to 1:0.73 and 1:0.82 to 1:0.85. Thus the former are more elongate than those of the *Boonea* species studied (Robertson 2012b), perhaps because the former species is so small. The capsule wall of both *Fargoa* species is thicker at one end than the other.

Egg sizes

Eggs of *Fargoa dianthophila* were 53 to 57 µm in diameter, and of *F. bartschi* 55 to 60 µm. No polar bodies were observed.

Embryos and veligers

Late embryos are considerably larger than the eggs but still small. Hatching veliger shell diameters in *Fargoa dianthophila* were 120 to 132 µm, and 129 to 158 µm in *F. bartschi*. The dark and opaque "PMO" (Robertson 1985, 2012a, 2012b) and paired statocysts were conspicuous at this stage, and an operculum and pedal bristles were present. Tentacles and eyes were lacking (Fig. 4). (Appearances of the tentacles and eyes are preludes to metamorphosis in *Boonea*.) In both *Fargoa* species there is a tiny, unpigmented

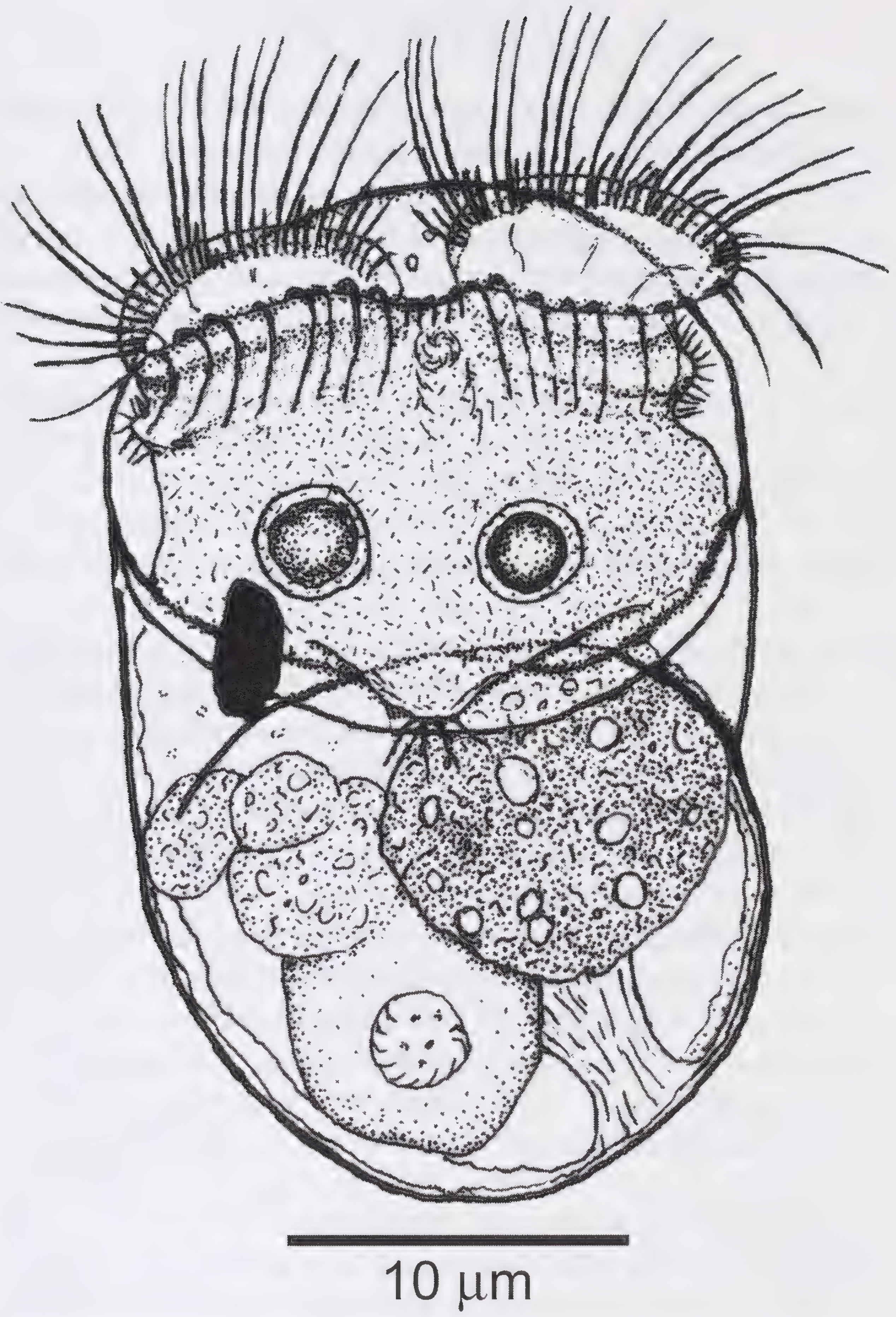

Figure 4. *Fargoa dianthophila.* Newly-hatched swimming planktotrophic veliger. Woods Hole. Note the nearly isostrophic larval shell and the small velum that does not reflect a short planktonic stage.

velum with two lobes subequal in size. The few swimming veligers available in the laboratory were kept in unfiltered seawater, and no attempt was made to rear them. One *F. bartschi* larva swam for nine days without growing and then died.

DISCUSSION

Although they have C-type protoconchs, both species of *Fargoa* appear consistently to be planktotrophic. Fragmentary data (Lebour 1932) suggest that one other C-type species may be lecithotrophic, but as yet there appear to be no data supporting the idea that C-type protoconchs in other pyramidellid genera ever reflect "direct" development. Thorson (1946) was wrong about *Brachystomia* "*scalaris*", his data showing that it is planktotrophic (Fig. 1).

There was no previous knowledge of the eggs or larvae of *Fargoa*. The velar lobes are tiny, which disagrees with Thorson‘s surmise (1946: 206) that a "small velum seems to indicate that pelagic life is very short." I have speculated (Robertson 1986) that the planktonic stages of the two *Fargoa* species last as long as two or three weeks. This is near the upper end of the range actually recorded for planktotrophic *Boonea bisuturalis* in the laboratory (Robertson 2012b). The range from hatching size to size at metamorphosis is relatively much greater in *Fargoa.*

Fargoa bartschi shows major geographical variation in maximum attained shell and spermatophore sizes. Do these also affect egg sizes and lengths of the pelagic stage? Note that in *Boonea* "*impressa*" smaller mature individuals perhaps lay slightly smaller eggs (Robertson 2012b).

The egg capsule wall morphologies of *Fargoa* and *Boonea* appear to differ, in the former being thicker at one end. However, this appears to vary intraspecifically in some Danish *Brachystomia* "*scalaris*" (Fig. 1; Robertson 2012b, Figs. 7 and 10). The capsules of both *Fargoa* species are more elongate than those of the *Boonea* species studied. They perhaps are squeezed into this shape in the smaller animals.

Clark and Goetzfried (1978: 291–292, fig. 5) believed that in planktotrophic sacoglossans and nudibranchs adult size controls egg volume and the number of eggs per egg mass. There may be a similar trend for small pyramidellids to lay the smallest and fewest eggs. The two *Fargoa* species (1.8 to 4 mm long), Danish and Swedish higher salinity *Brachystomia* "*scalaris*" (about 4 mm long), and *Boonea omaensis* (Nomura, 1938) (< 5 mm long) appear to have the smallest known pyramidellid eggs, respectively 53 to 60 µm (this paper), 55 µm and much higher (Fig. 1 and Robertson 2012a), and 60 µm (Amio 1959, as *Odostomia* (*Evalea* A. Adams, 1860)). The scale line for the illustrated early embryos of the last species indicates a smaller size. Judging by this, *B. omaensis* must be planktotrophic. A possible discrepancy: Ponder (1987: 19–24) recorded an egg "reaching about 0.05 mm in diameter" in *Longchaeus acus* (Gmelin, 1791), as *Pyramidella* Lamarck, 1799. This is the largest pyramidellid species (Laseron 1959: 191–192, fig. 20, as *Voluspa maculosa* (Lamarck, 1822)), up to 53 mm long and with about 20 whorls. This is the smallest recorded pyramidellid egg, but the report seems to have been based on developing oocytes observed histologically in the ovotestis (the "spawn [was] unknown"). Ponder did mention that the protoconch was heterostrophic and "minute." Systematic nomenclature in this paragraph follows Hori (2000).

In other "opisthobranchs", the smallest eggs are not necessarily laid by the smallest adults. Judging by data compiled by Jensen (2001), the smallest known precisely measured "opisthobranch" egg may be that of the plakobranchid sacoglossan *Elysia trisinuata* Baba (1949: 132, pl. 9, figs. 30, 31),

Table 1. Major reproductive features of *Fargoa dianthophila* and *F. bartschi* at Woods Hole, Massachusetts. Ranges, means, and sample sizes (in parentheses).

Species	*F. dianthophila*	*F. bartschi*
Eggs per egg mass	29–38	15–246[1]
Average (sample size)	33.5 (2)	122 (5)
Egg capsule lengths (µm)	137–158	165–193
Average (sample size)	149.0 (10)	179.4 (7)
Egg diameters (µm)	53–57	55–60[2]
Average (sample size)	54.7 (20)	(2)
Oviposition to hatching (days)	about 7	about 4–8
Hatching shell diameters (µm)	120–132	129–158
Average (sample size)		141.8
Veliger planktonic (days in lab)		> 9
Reproductive mode	Planktotrophy	Planktotrophy

[1] The upper number doubtless reflects several or many congealed masses.

[2] "ca. 87 µm" in Robertson (1996: table 1, 19A) was an error.

a slug 5 to 30 mm long (37 mm according to the figure magnification), not small. Its egg diameter was reported by Hamatani (1967) as 46 µm (but his scale line indicates about 53 µm). The smallest nudibranch eggs known to me apparently are those of the facelinid aeolid *Favorinus branchialis* (Rathke, 1806). These were reported by Haefelfinger (1962: 314) to be about 40 µm in diameter, each contained in an oval capsule 60 µm long and 45 µm wide. This slug attains lengths of 7 or 22 mm (Schmekel and Portmann 1982: 201), which again is not small.

The two *Fargoa* species have the same north Floridian disjunction in their geographical distributions as *Boonea* and many other non-tropical marine animals in that area (Robertson 2012b). Do they parallel *Boonea* "*impressa*" in having different intraspecific (?) egg sizes and modes of larval development in the Gulf of Mexico? In brackish water anywhere, do they have eggs of different sizes? Do small eggs, low egg numbers per mass, and small hatching sizes result from small adult sizes in some *Fargoa* species? Are there species in the genus with shells longer than 4 mm? Does the genus *Fargoa* consistently have relatively large C-type protoconchs resulting from relatively long planktonic stages? Is the genus invariably planktotrophic?

ACKNOWLEDGMENTS

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The influence of emersion on the rate at which *Nucella lamellosa* (Muricidae) consumes prey in the laboratory

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Abstract. *Nucella lamellosa* (Gmelin, 1791) is a phenotypically plastic species that preys on barnacles and occurs intertidally and subtidally along the north-eastern Pacific coast. This laboratory experiment tests the hypothesis that daily episodes of emersion impose a greater stress than daily episodes of limiting food in *N. lamellosa*. In this experiment, individuals of *N. lamellosa* were housed in containers with a rock covered with barnacles. In one experimental treatment, I removed snails from their barnacle-covered rocks and placed them on a counter for five hours each day. In another treatment, I placed the snail-and-rock assemblage on a counter for five hours each day. In a final experimental treatment, I kept the snails submersed, but placed the barnacle-covered rocks in an empty sea table for five hours each day. A control treatment kept individuals constantly submersed with continuous access to food. To account for the effect of handling the snails, individuals in a manipulation control were picked up and then immediately returned to their rocks. When individuals of *N. lamellosa* were removed from the water without their barnacle-covered rocks, they ate significantly less than snails in either control group and significantly less than the snails that were constantly submersed but without food for five hours a day. This result suggests that daily episodes of emersion are more stressful than daily episodes of limiting food.

Key words: submersion, barnacles, feeding behavior, desiccation, intertidal

Gastropods that live in the intertidal zone are subject to a variety of physical stresses. The predatory genus *Nucella* Röding, 1798 is appropriate for studying the impact of these stresses because individuals in the genus adjust their foraging habits and growth rates when they encounter stresses like wave activity (Etter 1996), predators (Bourdeau 2009, Freeman and Hamer 2009, Large *et al.* 2011), and elevated water and aerial temperatures (Bertness and Schneider 1976, Kuo and Sanford 2009, Yamane and Gilman 2009, Carrington and Kull 2011). However, the effect of emersion on the rate at which individuals of *Nucella* consume prey has yet to be studied.

It appears that the daily stress of tidal emersion impacts one species of *Nucella* in particular: *Nucella lamellosa* (Gmelin, 1791). *Nucella lamellosa* has both intertidal and subtidal populations. The individuals in the subtidal populations can get quite large with shell lengths commonly reaching 10 cm, but it is unusual for intertidal individuals to have shells that are longer than 5 cm (Kincaid 1957). Kincaid (1957) hypothesized that this size difference is due to the fact that intertidal individuals do not eat during low tide, and that subtidal individuals eat constantly. However, the impact of emersion on the rate of food consumption has not been formally tested. Tidal emersion could also impact the rate of food consumption because of other factors, such as high air temperatures and an increase in desiccation. A controlled laboratory experiment can quantify the effect of emersion, but also separate the effect of emersion from periods without access to food.

Nucella lamellosa is a phenotypically plastic species that occurs intertidally and subtidally along the eastern Pacific coast, from the Bering Sea to Monterey, California. Abundant and easy-to-collect, *N. lamellosa* specializes on barnacles as prey (Kincaid 1957) and grows quickly in the laboratory (Bourdeau 2009, 2010, 2011), so it is ideal for laboratory experiments.

In this study, in order to specifically address emersion as opposed to other environmental variables that change with the tides, I tested the hypothesis that daily episodes of emersion in the laboratory impose a greater stress than daily episodes of limiting food in *Nucella lamellosa*. I predict that the stress of emersion leads to a significant decrease in barnacle consumption.

MATERIALS AND METHODS

Collections

Individuals of *Nucella lamellosa* were collected from False Bay (48°28′N, 123°03′W), San Juan Island, Washington during a night tide on 30 January 2010 from large rocks and overhangs that were covered with barnacles. Smaller, younger individuals of *N. lamellosa* are more sensitive to environmental variables that affect the rate of growth, such as the rate of consuming prey, because they grow faster than larger, older individuals (Spight 1972). Therefore, I collected juveniles with shell lengths between 19 and 28 mm. Because the individuals in this locality coexist with crabs of the species *Cancer productus*, the snails had the thick-shelled phenotype that lacks lamellae (Kincaid 1957, Appleton and Palmer 1988). Once in the laboratory, snails were maintained in running seawater tables for three days before the experiment.

Treatments

All of the experiments were conducted at the same time at the University of Washington's Friday Harbor Laboratories, San Juan Island, Washington from February 2, 2010 to February 27, 2010. Each snail was maintained in its own 1.15 L (16 × 8 × 9 cm) plastic container with ambient light conditions. Each container held one or two small rocks covered with barnacles (*Balanus glandula*) so that the individuals of *Nucella lamellosa* always had an abundant supply of food. The containers were distributed randomly among six sea tables, each of which contained a holding tank that was constantly refreshed with running seawater. The holding tanks contained heaters to elevate sea water from winter to summer temperatures, which are associated with higher growth rates (Price, pers. obs.). Water temperature in the holding tanks was recorded every half-hour throughout the day with TidBiT water temperature data loggers (Onset Corporation), and averaged 12.39 ± 0.81 °C over the course of the experiment. Seawater left the holding tanks and entered each container through 2.38 mm inner diameter PVC tubing. Water exited each container through a hole in the lid. Containers were rinsed in sea water when there was evidence of fouling.

Each individual of *Nucella lamellosa* was randomly assigned to one of five treatments. Two of the treatments were controls. The controls kept the snails constantly submersed to mimic the subtidal populations that Kincaid (1957) indicated had constant access to food. The primary control kept animals constantly submersed with constant access to food. However, because all of the other treatments involved moving the snails, I needed to determine whether handling during the course of the experiment impacted the rate at which snails consumed barnacles. Therefore, in a second control treatment, I picked up each individual (which was usually situated on the barnacle-covered rock in its container), took it out of the container, then immediately returned it to the same place in the container from which it was removed.

The experimental treatments varied either the availability of food for submersed individuals or whether the individuals were emersed. In one experimental treatment, I removed snails from their barnacle-covered rocks and placed them on a counter for five hours each day, with 4 or 5 cm in between them. This made it easy to find snails that crawled away from their rocks while they were emersed, and it prevented snails from getting washed into pipes in the sea table. The barnacle-covered rock remained submersed in the mesocosm. In another experimental treatment, I placed the snail-and-rock assemblages on a counter top about 4-5 cm apart from each other for five hours each day. In the final experimental treatment, I kept the snails submersed; the barnacle-covered rocks were placed in a sea table, in which they were partially submersed with seawater splashing them. Air temperature during the time of emersion averaged 20.68 ± 2.14 °C. The experimental manipulations occurred from 7:30 am to 12:30 pm.

Individual snails were labeled with bee tags that were attached to the shells with cyanoacrylate adhesive. This labeling made it easier to return them to the appropriate container and to track individual consumption rates.

Most treatments were replicated ten times, although the treatment that emersed individuals of *Nucella lamellosa* with barnacles had only nine replicates.

Barnacle consumption

Empty barnacle tests were removed from each barnacle-covered rock at the beginning of the experiment before the rock was added to the container. Thus, all of the empty tests at the end of the experiment belonged to barnacles that died during the experiment. To estimate the number of barnacles that died due to predation by *Nucella* and those that died for other reasons, I maintained ten replicates of a barnacle control treatment. In this treatment, the barnacles were maintained in containers identical to those described above, constantly submersed but without *Nucella lamellosa.* At the end of the experiment, I calculated the average number of barnacles that died in the control treatment (4 ± 0.6 SE) and subtracted this number from the number of barnacles that died in the treatments with snails.

Statistical analyses

All analyses were conducted in R (R Development Core Team 2011). Because growth rate varies with size (Price, pers. obs.), and because consumption rate is presumably correlated to growth, I tested whether initial size affected consumption rate. However, there was such little variability in initial size both within and across treatments, that initial shell length did not affect the outcome; an ANCOVA analysis indicated that neither shell length nor the interaction between shell length and the number of barnacles consumed were significant. Thus, ANOVA, and not ANCOVA, was an appropriate analysis to identify significance across treatments (Sokal and Rohlf 1995). The Tukey High Significant Difference test was used as an *ad hoc* method to identify differences between treatments (Quinn and Keough 2002).

RESULTS

Daily episodes of emersion significantly reduced the rate at which individuals of *Nucella lamellosa* ate barnacles. Snails in the control treatment consumed significantly more barnacles than snails in either of the treatments that experienced emersion (* in Fig. 1, Table 1). Snails in the manipulation control ate significantly more than snails that were emersed without barnacles for five hours (** in Fig. 1). The snails that were emersed without barnacles for five hours a day ate significantly less than those that were constantly submersed but separated from the barnacles for five hours a day (*** in Fig. 1). Some of these emersed snails did not eat any barnacles (Fig. 1). Other pair-wise comparisons were not significant at $\alpha = 0.05$ (Table 1).

DISCUSSION

The fact that individuals of *Nucella lamellosa* who were emersed without their barnacles consumed significantly fewer barnacles than snails in the control treatment supports the hypothesis that daily episodes of emersion in the laboratory impose a greater stress than daily episodes of limiting food. However, during the 25 days of this experiment, removing the food source for five hours a day did not reduce the total number of barnacles consumed (Fig. 1). Thus, individuals of *N. lamellosa* made up the deficit from five hours without food once their food source became available. Neither control treatment differed significantly from the treatment in which snails were submersed but subjected to daily episodes without food.

The experimental design assumed that emersion does not significantly change the quality of barnacles as prey. The barnacles were constantly submersed within the mesocosms when snails were constantly submersed with them (control in Fig. 1), when snails were submersed with them except during a brief interval during which the snails were handled (manipulation control), when the snails were emersed for five hours without their barnacles (emersed, no barnacles), and when the barnacles were constantly submersed without snails for the duration of the experiment (barnacle control). Barnacles in the barnacle control had the highest rate of survival. Barnacles in the emersed, no barnacles treatment had the second highest rate of survival; snails in that treatment ate significantly less than snails in the control and manipulation control. Thus, the main result of this experiment is drawn by comparing treatments in which the barnacles were treated the same way.

The results from the remaining two treatments are harder to interpret, because the barnacles were not constantly submersed. Barnacles were only partially submersed in the treatment in which the snails were constantly submersed and the barnacles removed from the mesocosms (submersed, no barnacles in Fig. 1). However, the survival of barnacles was not significantly different between this treatment and either of the controls (Fig. 1, Table 1), suggesting that the survival of barnacles is not significantly impacted by partial submersion. Barnacles were completely emersed in the treatment in which the snails were emersed for five hours with the barnacle-covered rock (emersed). The number of barnacles that died in this treatment was significantly less than the number that survived in the manipulation control, and significantly more than died in the emersed, no barnacles treatment. Because none of the controls replicated complete emersion of barnacles for five hours, it is unclear how much of this result is due to the emersion of barnacles, and how much

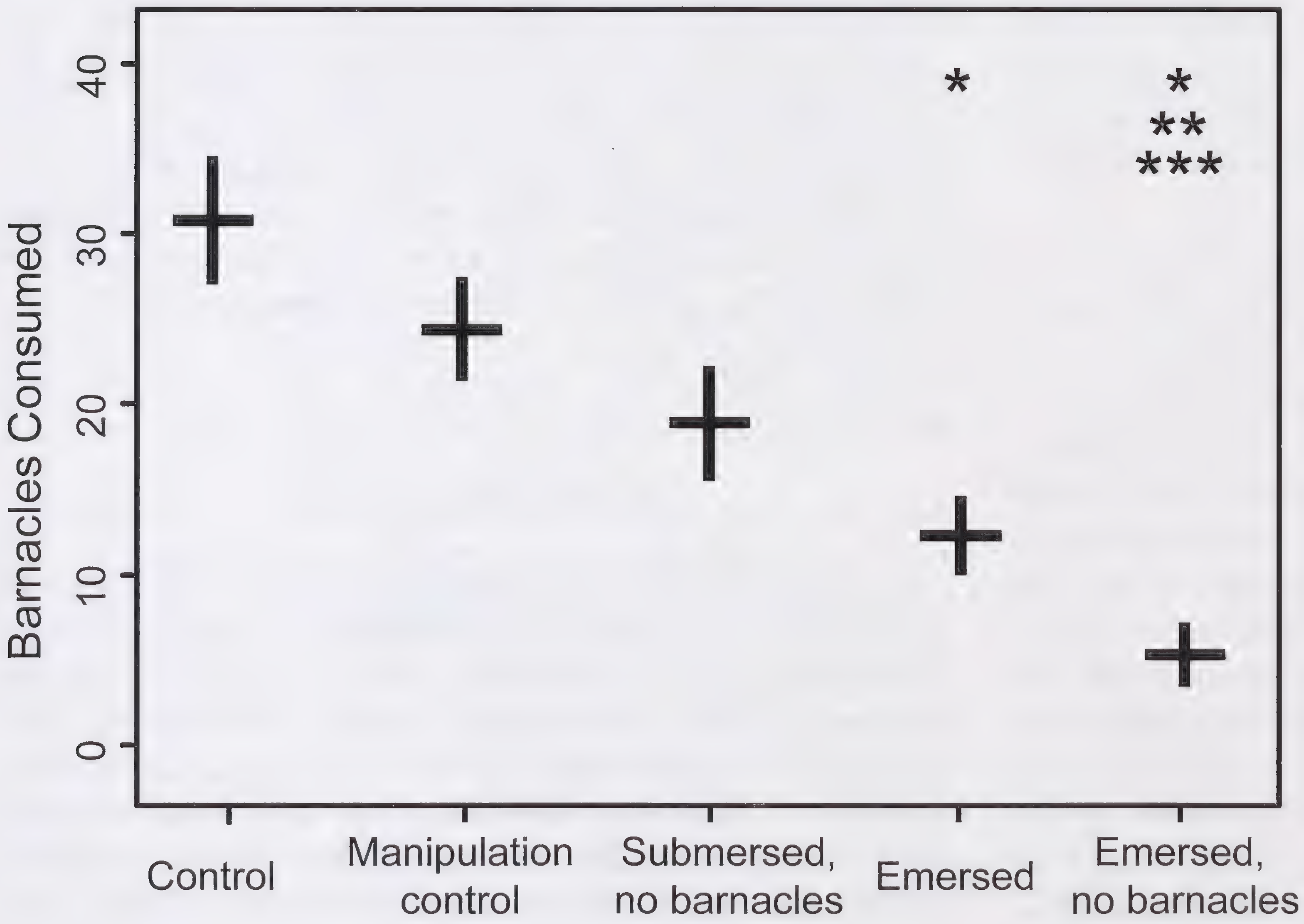

Figure 1. The number of barnacles consumed in each of the treatments with the mean (horizontal bars) and standard error (vertical bars). Asterisks highlight significant results from the Tukey High Significant Difference test ($P < 0.001$, Table 1). *, significantly different from the control. **, significantly different from the manipulation control. ***, significantly different from five hours of submersion without barnacles.

Table 1. *P*-values from the Tukey High Significant Difference Test. * indicates significant at $\alpha = 0.05$.

	Manipulation	Submersed, no barnacles	Emersed with barnacles	Emersed, no barnacles
Control	0.55	0.051	< 0.001*	< 0.001*
Manipulation control	-	0.69	0.055	< 0.001*
Submersed no barnacles	-	-	0.55	0.019*
Emersed with barnacles	-	-	-	0.50

is due to the emersion of snails. Future work should control for the effect of emersion on barnacle prey.

The impact of emersion on the rate of barnacle consumption

Daily episodes of emersion seem to be more stressful than daily episodes of limiting food in individuals of *Nucella lamellosa*. Individuals eat the least amount when emersed and separated from their food source (Fig. 1).

The impact of emersion was particularly pronounced in the treatment in which snails were emersed without their barnacle-covered rocks. This treatment was so stressful that three of the individuals did not eat at all. During emersion, the snails in this treatment were placed far away from each other. However, during low tide in the field, individuals of *Nucella lamellosa* are either loosely aggregated under rocks, or they are attached to barnacles. Both of these habits may offer some protection from the effects of emersion by minimizing the effects of desiccation. The act of aggregating may be critical for avoiding desiccation by increasing the surface area where moisture can be trapped around the snails (Chapman and Underwood 1996).

In order to reach its prey, an individual of *Nucella lamellosa* uses its radula to puncture a hole in a barnacle's operculum (Kincaid 1957), and then presumably, as in *N. lapillus*, it remains situated over the barnacles' tests until ingestion is complete (Hughes and Burrows 1994). In *N. lapillus* ingestion can take up to 26 hours (Dunkin and Hughes 1984). The number of barnacles eaten in the manipulation control, the submersed experimental treatment, and the emersion without barnacles treatment may have been influenced by the fact that feeding was interrupted when the snails were handled. The daily stress inflicted on these animals interrupted their feeding regimen. It is unclear whether or not the snails are actively eating while emersed. If they cease to eat during low tide, then the snails that remain on barnacles during low tide in the field may be in the middle of a feeding cycle, and it may be easier for them to resume eating when the tide returns if they remain undisturbed.

Implications for tidal emersion

The results presented here imply that daily tidal emersion imposes a serious stress, and that the period of submersion following emersion is not sufficient to compensate for the deficit in consumption before the next tidal cycle begins. The experimental treatments described here did not replicate tidal emersion, but instead analyzed the effect of daily emersion—at the same time of day—on *Nucella lamellosa* over 25 days. In the field, tidal emersion is a slow process, and desiccation is not as extreme because water stays in small pools and cracks during low tide. Nonetheless, these results indicate that emersion carries a cost: it slows rates of consumption. The results are consistent with Kincaid's (1957) hypothesis that individuals of *N. lamellosa* in intertidal populations are smaller than individuals in subtidal populations because tidal emersion is stressful.

Because aerial temperature during low tide is beginning to increase, intertidal organisms will be subject to more thermal stress and more desiccation (Helmuth *et al.* 2002, Helmuth *et al.* 2006, Kuo and Sanford 2009). Future research may explore whether individuals of *Nucella lamellosa* respond to tidal cycles of emersion similar to the way they respond to daily episodes of emersion in the lab. If they do, then the rate of prey consumption in individuals of *Nucella lamellosa* will decrease when they are subjected to emersion, which could ultimately decrease fecundity because there will be less energy available for reproduction.

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Epibionts on *Aequipecten tehuelchus* (d'Orbigny, 1846) (Pectinidae) in shelf waters off Buenos Aires, Argentina

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Abstract. *Aequipecten tehuelchus* (d'Orbigny, 1846) is a commercial resource, distributed from Rio de Janeiro (Brazil) to Golfo Nuevo (Argentina), on sandy and muddy bottoms. In Argentina, the major banks of exploitation are located in the gulfs of San José and San Matías. This study represents the first ecological study of *A. tehuelchus* in shelf waters off Buenos Aires, Argentina. Eighteen epibiont taxa were registered on the individuals analyzed. Polychaetes were the most frequent epibiotic organisms (82%), including Serpulidae (Rafinesque, 1815), *Phyllochaetopterus socialis* (Claparède, 1869), *Idanthyrsus armatus* (Kingberg, 1807) and *Chaetopterus antarcticus* (Kingberg, 1866). *Ostrea puelcheana* (d'Orbigny, 1842), *Balanus* cf. *amphitrite* (Darwin, 1854) and solitary ascidians were found on less than 30% of the sampled individuals. Bryozoa and Porifera were scarcely represented. *Phyllochaetopterus socialis, I. armatus* and *Balanus* cf. *amphitrite* were most frequent on the upper (left) valve. This study nearly doubles the number of epibionts identified and mentioned on *A. tehuelchus* in Patagonian Gulfs. Three individuals of the commensal pea crab *Tumidotheres maculatus* (Say, 1818) were found inside three different specimens of *A. tehuelchus*. One left scallop valve was burrowed into by the parasitic polychaete *Polydora* (Bosc, 1802).

Key words: Epibiosis, scallop, SW Atlantic Ocean

Epibiosis, a common phenomenon in the marine environment, is the association between epibionts (organisms growing attached to a living surface) and basibionts (organisms that provide substrate to the epibionts). This association creates a complex network of benefits and disadvantages for both organisms (Wahl 1989, 2009). Filter-feeding epibionts possibly profit from the nutrient currents created by certain hosts (Laihonen and Furman 1986), but it has been suggested that energy flow between epiphytic partners may occasionally come to benefit of the host (Harlin 1973).

Frequently epibiotic cover can play a protective role (Wahl 1989) or reduce the predation of basibionts (Ward and Thorpe 1991) and in this form the danger of falling victim to predators of the substrate organism is one of the greatest hazards in an epibiont's life (Cuomo *et al.* 1985). However, fouling organisms (e.g. Porifera, Cnidaria, Serpulidae, Bryozoa) can compromise movement capabilities of hosts (Dixon *et al.* 1981, Cuomo *et al.* 1985) reducing possibilities to escape from predators (Cerrano *et al.* 2009) or reducing larval dispersal feeding on newly released larvae (Cerrano *et al.* 1998). In addition, the epibionts cause difficulty in opening and closing the valves, thus affecting the feeding and breathing in bivalves (Uribe *et al.* 2001). Epibiont organisms are often themselves filter feeders so they may compete with the hosting species for food resources (Claereboudt *et al.* 1994).

Epibiotic invertebrates encrust the valves of scallops around the world, mainly in areas with predominant soft bottoms (e.g. Ward and Thorpe 1991, Claereboudt *et al.* 1994, Rosso and Sanfilippo 1994, Sanfilippo 1994, Cerrano *et al.* 2006, Schejter and Bremec 2007a). The tehuelche scallop, *Aequipecten tehuelchus* (d'Orbigny, 1846), is a commercial resource, distributed from Rio de Janeiro (Brazil) to Golfo Nuevo (Argentina), on sandy and muddy bottoms (Olivier *et al.* 1970). In Argentina, artisanal fishery by means of diving has developed mainly at the San José and San Matías Gulfs (42° S). Numerous studies on the biology, anatomy, physiology, population dynamics and fishery have been carried out on this pectinid (see Ciocco *et al.* 2006). However, most studies were conducted in Patagonian gulfs and there are only faunistic records outside of this area (e.g. Carcelles 1944, Castellanos 1970). During 2002, a monitoring program was conducted between 39–40°S and nearly 45 m depth to locate potentially exploitable beds of tehuelche scallop. During those expeditions, heterogeneous areas were sampled, including patches with dominance of eunicid polychaetes, ascidians, mussels, and also some dominated by the tehuelche scallop (Schejter and Bremec 2007a). In this paper we aim to identify and quantify epibiotic richness on *A. tehuelchus*. This research contributes to the understanding of the benthic community structure from soft bottoms off Buenos Aires, information also useful in a fisheries context.

MATERIALS AND METHODS

The study material comes from six benthic community samples taken in 2002 between 39°01′–39°40′S and 58°40′–60°21′W on board the FV "Erin Bruce" with otter trawls. Additionally two other samples collected with dredges at 38°26′S–57°40′W and 38°28′S–57°41′W in 2004 by the RV "Capitán Cánepa" INIDEP were analyzed (Fig.1). All samples were frozen on board and then preserved in 5% formaldehyde. Two hundred and twenty seven scallop individuals of different sizes were examined. In the laboratory, the epibionts on *Aequipecten tehuelchus* were identified to the lowest possible taxonomic level. Presence-absence and quantitative data of epibionts were recorded for lower (right) and upper (left) valves. The McNemar test for paired proportions (Sokal and Rohlf 1979) was used to establish preferences of the epibionts for any of the scallop valves.

Valves were arbitrarily divided into seven areas to establish the preference of the epibionts for any region following the procedure modified after Ward and Thorpe (1991) and Sanfilippo (1994) by Schejter and Bremec (2007a) (Fig. 2A). For this analysis, the individuals greater than 50 mm were considered; maximum shell height was measured with calipers to the nearest mm.

RESULTS

Scallops ranged from 40 to 88 mm total shell height (average = 73mm). Eighteen epibiont taxa were registered on the individuals analyzed (Table 1; Fig. 2). Ninety one percent of the examined tehuelche scallops showed epibionts. Polychaetes were the most frequent epibiotic organisms (82%). *Serpula* sp. was present on 75% of the sampled scallops, followed by Spirorbinae (28%), *Phyllochaetopterus socialis* (21%), *Idanthyrsus armatus* (11%) and *Chaetopterus antarcticus* (0.4%). *Serpula* sp. encrusted both valves, *P. socialis* and *I. armatus* showed a preference for the upper (left) valve (McNemar test: left $\chi^2 = 6.56$ and $\chi^2 = 5.5$ respectively, $P < 0.05$), while Spirorbinae was more frequent on the lower valve (right) (McNemar test: right $\chi^2 = 13.13$, $P < 0.05$) (Fig. 3).

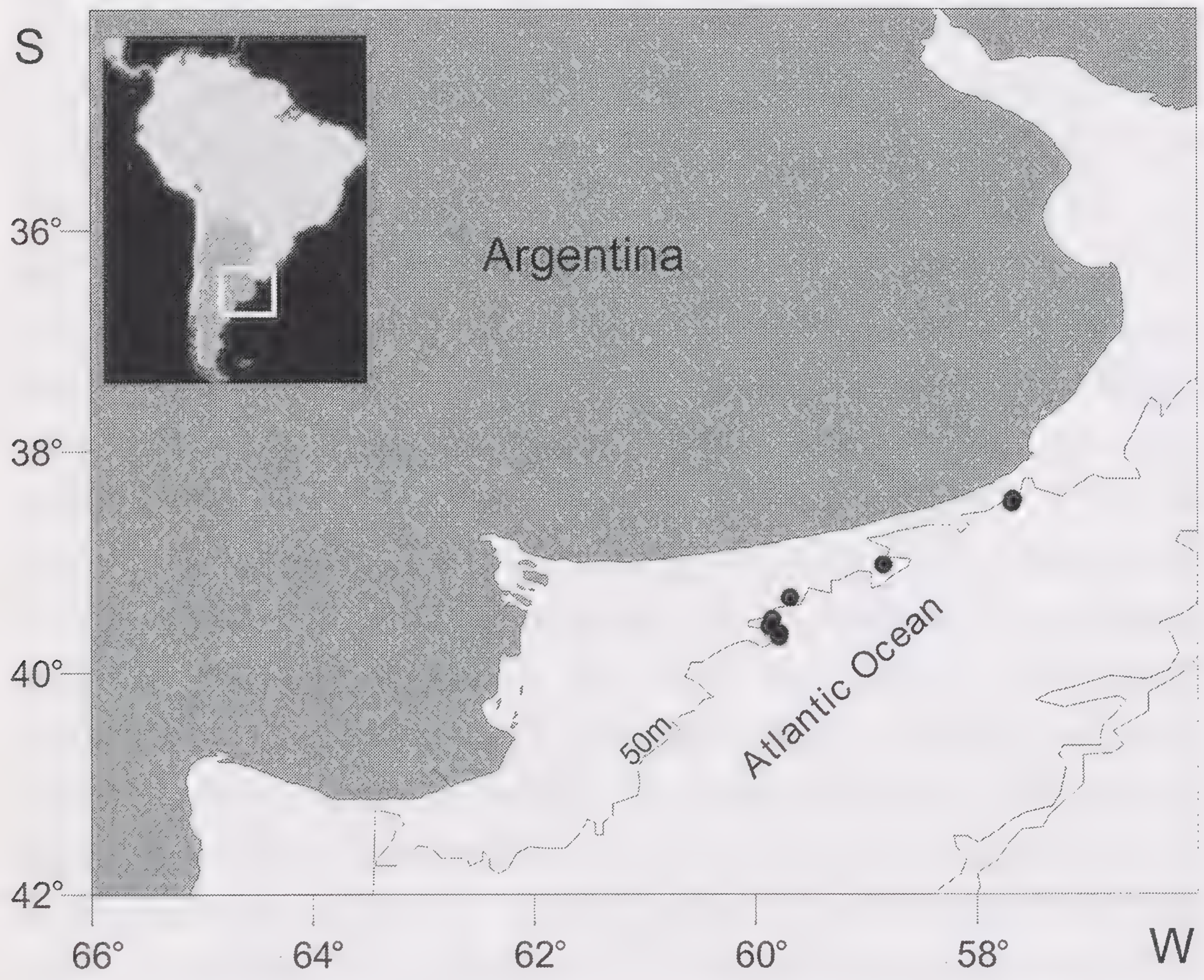

Figure 1. Map of the sampling area, Argentina. The dots (●) represent the trawling sites.

In general, polychaetes frequently settled on the seven regions of the valves. *Idanthyrsus armatus* presented low frequency of occurrence, 4.35%, in regions 6 and 7 (auricular area) and *Phyllochaetopterus socialis* showed 7.9% in region 4 (Fig. 4).

Solitary ascidians were found on 27.7% of the sampled scallops, in both valves and in all regions (Figs. 3, 4).

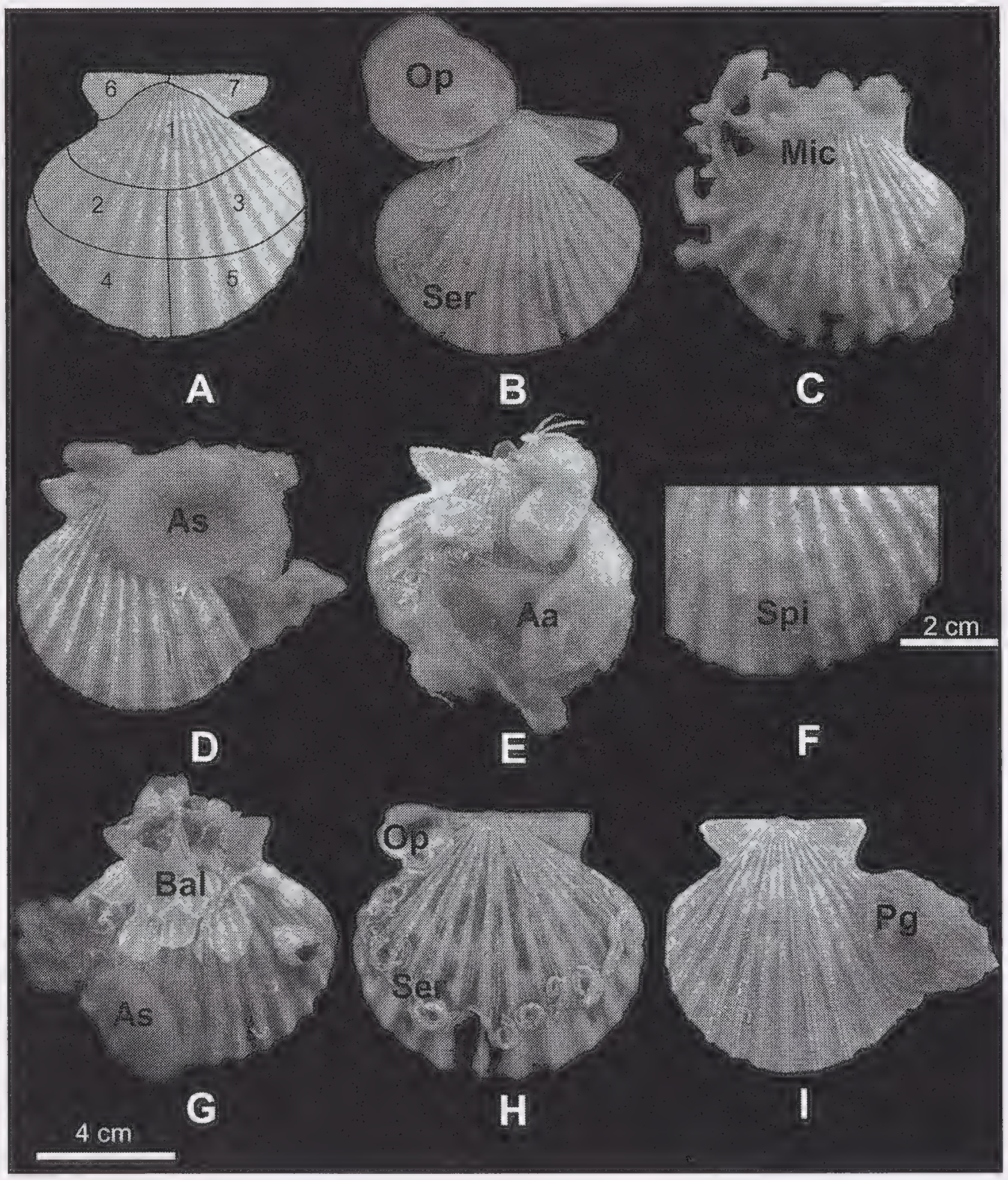

Figure 2. *Aequipecten tehuelchus* epibionts in the study site. **A**, diagram showing the division of each valve into 7 arbitrary regions. **B**, one individual of *Ostrea puelchana* (**Op**) and some *Serpula* sp. tubes (**Ser**). **C**, Microcionidae (**Mic**). **D**, Ascidiacea (**As**). **E**, several individuals of *Ascidiella aspersa* (**Aa**). **F**, several tubes of Spirorbinae (**Spi**). **G**, *Balanus* cf. *amphitrite* and Ascidiacea. **H**, *Ostrea puelchana* and *Serpula* sp. tubes. **I**, *Paramolgula gregaria* (**Pg**).

Table 1. List of epibionts on *Aequipecten tehuelchus* in the study site.

Taxa
PORIFERA
Microcionidae
Dictyoceratida
ANNELIDA
Eunice juvenile
Chaetopterus antarcticus Kingberg, 1866
Phyllochaetopterus socialis Claparède, 1869
Idanthyrsus armatus Kingberg, 1807
Polydora sp.
Serpula sp.
Spirorbinae
MOLLUSCA
Ostrea puelchana d'Orbigny, 1842
ARTHROPODA
Tumidotheres maculatus (Say, 1818)
Balanus cf. *amphitrite* Darwin, 1854
BRYOZOA
Bryozoa unidentified
CHORDATA
Paramolgula gregaria (Lesson, 1830)
Cnemidocarpa robinsoni Hartmeyer, 1916
Ascidiella aspersa (Müller, 1776)
Pyura legumen (Lesson, 1830)
Ascidiacea

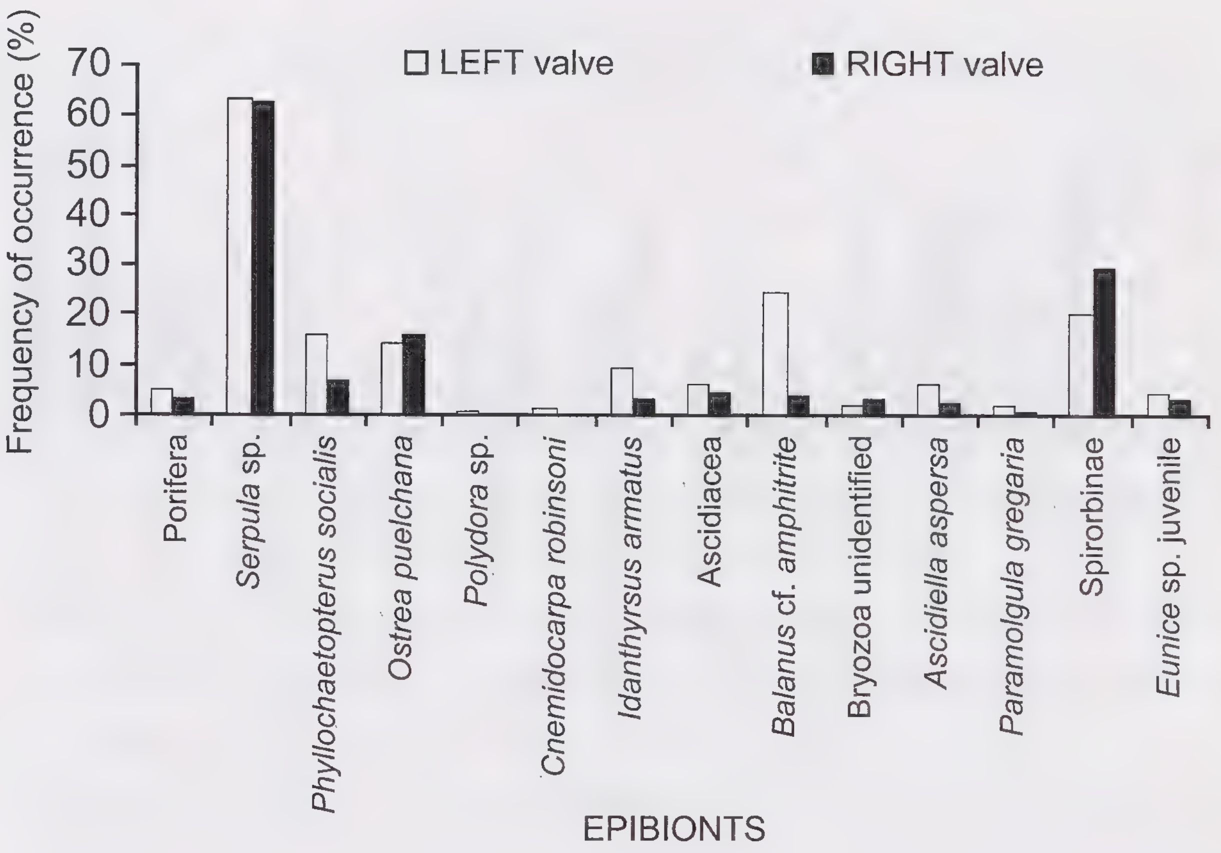

Figure 3. Frequency of occurrence of epibionts in both valves of *Aequipecten tehuelchus*, Buenos Aires, Argentina.

Oysters, *Ostrea puelchana* (d'Orbigny, 1842), were found on 24.7% of the sampled individuals, in all regions, and showed similar occurrence on both valves (14% on left valve and 16% on right valve) (Figs. 3, 4).

Cirripeds were found on 24% of the sampled scallops; they were present on 47 (24.5%) upper valves and on only 7 (3.65%) lower valves (Fig. 3). *Balanus* cf. *amphitrite* showed a preference for the upper (left) valve (McNemar test: left $\chi^2 = 30.42$; $P = 0.05$), with presence in all regions, conspicuously in region 1 (Fig. 4).

Bryozoa (3.5%) and Porifera (7.9%) were uncommon epibionts and were present on both valves and all regions. Region 1 was conspicuously encrusted by Porifera (81%) (Fig. 4).

Two males (6.5–6.6 mm carapace length) and one female (11 mm carapace length) of the pea crab *Tumidotheres maculatus* (Say, 1818) were found inside three different specimens of *Aequipecten tehuelchus*. One left scallop valve was burrowed into by the parasitic polychaete *Polydora* sp.

DISCUSSION

In the present study 91% of the *Aequipecten tehuelchus* examined off Buenos Aires province between 38–39°S and 45–50 m depth showed epibionts and the number of epibiotic taxa associated was 18. Previous information on the same scallop species from San Matías Gulf (41–42°S) at 50 m depth, mentions 10 taxa on almost all individuals (98%) (Olivier *et al.* 1970). In general, the main invertebrate groups encrusting *A. tehuelchus* are coincident in both areas, mainly serpulids, barnacles and oysters, however algae were found only in San Matías gulf. The species richness of epibionts associated with the scallops and the pattern of colonization reflects the life habits of scallops and varies between the pectinid species (Rosso and Sanfilippo 1991, Cerrano *et al.* 2006, Schejter and Bremec 2007a, b). We analyzed 227 individuals of tehuelche scallop and found 18 epibiotic taxa. Preliminary results considering only 158 individuals of tehuelche scallop showed the same number of epibiont taxa (Souto *et al.* 2008). Compared with studies in other pectinids around the world, *A. tehuelchus* presented a relatively low number of epibionts. Forty-nine taxa were associated with *Placopecten magallanicus* (Gmelin, 1791) in the lower Bay of Fundy (Canada), the sediment in this area is made up of a mixture of sand, clay, pebbles, boulders and cobbles (Fuller *et al.* 1998). At least 43 taxa were associated with *Adamussium colbecki* (Smith, 1902) in Antarctica (Bertolino 2004), region where seabed is characterized by pebbles of various sizes in shallow waters, changing to fine-grained, muddy sediments below 40–50 m in depth (Cerrano *et al.* 2006). Seventy epibiotic taxa were associated with *Zygochlamys patagonica* (King, 1832) in several banks distributed in soft bottoms of the Argentine Continental shelf (Schejter and Bremec 2007b, Lopez Gappa and Landoni 2009).

The number and composition of epibionts found in *Aequipecten tehuelchus* is coincident with that found in *Flexopecten felipponei* (Dall, 1922) by Schejter and Bremec (2007a) in the same sampling area. However the presence of

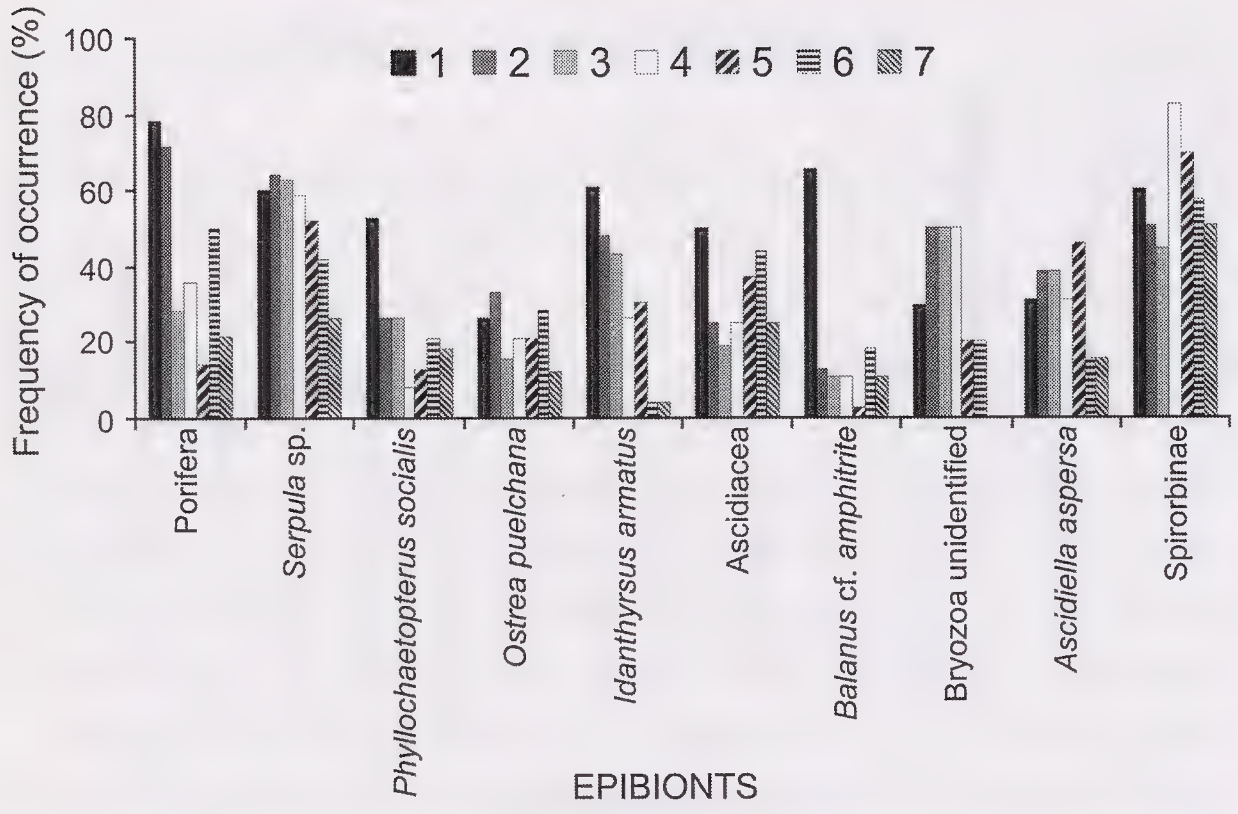

Figure 4. Frequency of occurrence of epibionts on each region of the valves of *Aequipecten tehuelchus*, Buenos Aires, Argentina.

Porifera was only recorded in the present study. Although sponges were found in few individuals (7.9%), when present, they reached important shell coverage. Sponges were scarcely recorded (< 5%) in the faunistic composition of the study area. Their presence on *A. tehuelchus*, but not on *F. felipponei* (Schejter and Bremec 2007a), could be related to the higher relative abundance of the former species in the study area. However, presence/absence of any kind of chemical compound, shell structure, competition and other interactions among epibionts could not be discarded as possible explanations of epibiotic taxa found (Wulff 2006). *Aequipecten tehuelchus* presents prominent ribs and riblets ornamented by lamellae, while the surface of *F. felipponei* is smooth.

Among other shell traits that affect potential colonization by epibiont organisms, shell size is particularly important. Larger shells usually support more individuals of a given fouling species (Rosso and Sanfilippo 1991, Gutierrez *et al.* 2003) and a greater richness of fouling species than smaller ones (Ward and Thorpe 1991). The total surface encrusted tends to increase progressively from the small scallops to the large ones (Rosso and Sanfilippo 1991). In our study, we analyzed 25 individuals ranging from 11 to 26mm. Most of these specimens showed both valves free of epibionts, with the exception of a few with low coverage of cirripeds on the upper valve. These scallops came from the northern sampling locations populated by *Mytilus edulis platensis* (d'Orbigny, 1846) banks and associated invertebrates species (Bremec and Roux 1997), while the heavily encrusted and bigger ones came from the southern sampling stations where the dominant species were *Aequipecten tehuelchus, Mytilus edulis platensis,* Ascidiacea *(Paramogula gregaria, Ascidiella aspersa, Cnemidocarpa robinsoni)* and *Eunice magellanica* (Schejter and Bremec 2007a). The variability of the sublittoral sediment types off Buenos Aires, with dominant soft bottoms and patches of hard substrates, establishes the existence of different benthic assemblages (Roux *et al.* 1993), all of them with a huge number of invertebrates capable of colonizing the surface of scallops (Bremec and Roux 1997, Schejter and Bremec, 2007a). Therefore, in agreement with previous studies, the low degree of epibiosis found in the present sampling of small scallops can be attributed to the small shell height of the specimens. Regarding the life habit of this species, the tehuelche scallop may do quick and brief swimming movements as escape responses to predators or other disturbances, but no information on the burrowing ability is available (Ciocco *et al.*, 2006). According to our results, this ability in *A. tehuelchus* seems to be reduced or absent, because epibionts heavily encrust the valves in high proportions.

In our study area, *Aequipecten tehuelchus* is one more example of the important role of mollusks as ecosystem engineers (Gutiérrez *et al.* 2003, Cerrano *et al.* 2006, Schejter and Bremec 2007a, b). This area of the Argentine Sea is frequently subjected to coastal hydrodynamic phenomena that remove sediments (see Bremec and Roux 1997). As a consequence, scallops provide substrate for the settlement and variety of niches available for different species of benthic invertebrates. In this sense, habitat heterogeneity is important for maintaining high species richness, given that a landscape of different habitat types should contain more species than homogeneous one (McLean 1983, Sousa 1985).

A preference for any valve was also detected in some epibionts. Spirorbinae was more frequent on the right valve, while the polychaetes *Idanthyrsus armatus* and *Phyllochaetopterus socialis* and cirripeds were more common on the left (upper) valves. This condition was observed in other pectinids and in general, differences could be attributed to competitive interactions, different strategies of colonization developed by the different groups, adaptations to the physical abrasion effects, and/or feeding strategies (Rosso and Sanfilippo 1991, Ward and Thorpe 1991, Sanfilippo 1994, Schejter and Bremec 2007a). *Idanthyrsus armatus* and *Balanus* cf. *amphitrite* showed similar and high frequency in all regions, except in valve regions 6 and 7; the small auricular areas would not be appropriate for the development of these relatively large species. Regions 2, 3 and 4 were conspicuously encrusted by Bryozoa. The same pattern was observed by Ward and Thorpe (1991) in *Chlamys opercularis* (Linnaeus, 1758), which presented a greater frequency of occurrence and coverage of Bryozoa in the central and peripheral regions of the valves. Sanfilippo (1994) observed that the distribution of Spirorbinae *Protolaeospira lebruni* (Caullery and Mesnil, 1897) was not homogeneous on the right valve of the Patagonian scallop, showing a greater concentration in the umbonal regions, since these areas are available for colonization for a longer period than the younger peripheral parts of the shell. Also, this epibiont was the unique that incrusted the shells with a size less than 35 mm. In our study, although not the only taxa on the auricles, Spirorbinae reached the highest frequency of occurrence in this region. This small settlement surface allows the development of relatively small species better than of larger ones.

One of the main problems in shellfish aquaculture and fisheries is the plague caused by the polychaete family Spionidae (McGladdery *et al.* 2006). The most common and harmful damage that they cause is called "mud blister", weakness in the valves and making them less resistant to predators and other pests (Ciocoo 1990). Spionidae infestation in valves of *Aequipecten tehuelchus*, reported for areas of North-Patagonian gulfs, reached percentages of occurrence greater than 10% (Diez *et al.* 2003). In Buenos Aires, the frequency of occurrence of Spionidae on *A. tehuelchus* was less than 1%, similar to the values found in *Flexopecten felipponei* (Schejter and Bremec 2007a). In addition, two valves with perforations caused by non-identified boring organisms were found. There are several marine organisms (algae, fungi, sponges, bivalves, polychaetes) that show an important bio-erosion activity on carbonate substrates in diverse habitats (e.g. Cerrano *et al.* 2001, McGladdery *et al.* 2006).

Crabs of the family Pinnotheridae occur in some commercially important bivalve mollusks and are often described as commensal or parasitic. In *Aequipecten tehuelchus*, the relationship with this pinnotherid is not commensal but parasitic and deleterious effects have been observed in infested scallops (Narvarte and Saiz 2004). In our study, only three crabs (two males and one female) were found inside three different specimens of *A. tehuelchus*

This study represents the first ecological study of *Aequipecten tehuelchus* in shelf waters off Buenos Aires, Argentina. Furthermore, we increased to nearly double the number of epibionts identified and mentioned on *A. tehuelchus* in Patagonian gulfs (Olivier *et al.* 1970).

The study sites have been prospected to assess bivalves standing stocks, mainly *Mytilus edulis platensis* and *A. tehuelchus.* Although commercial fishery of those species is not developed due to unprofitable catches, the area is currently subjected to fish and prawn demersal fisheries. This research gives additional information on benthic biodiversity and contributes to the understanding of the community structure from soft bottoms off Buenos Aires.

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Variability in the diet specialization of *Enteroctopus dofleini* (Cephalopoda: Octopodidae) in the eastern Pacific examined from midden contents

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Abstract. We examined variation in dietary specialization of *Enteroctopus dofleini* (Wülker, 1910), the giant Pacific octopus, from Puget Sound to the Aleutian Islands, as represented in midden remains. Dietary specialization was measured from midden contents as: species richness (R) and Cardona's niche breadth (regional indices), and proportional similarity of a midden to the regional sample (an individual index). We found an influence of items per midden and prey species maximum size on these indices. In Puget Sound, middens and common prey species were large, richness, R = 21 species, and individuals specialized more often than in other areas, typically on large prey species. In Saanich Inlet, British Columbia, middens were smaller, R = 9 species, and the large crab *Cancer productus* was common in nearly all middens, such that the population specialized rather than the individual. In Prince William Sound, Alaska, middens contained fewer individuals of smaller prey species, R = 52 species, and diet was generalized at both the population and the individual levels. Cardona's niche breadth ranged from .11 to .14 except in the Aleutian Islands, Alaska where it was higher (.30). Most individual *E. dofleini* were generalists, and dietary species richness was very high (R = 69 species overall). Specialists within a generalist population were common only where middens and dominant prey were both large.

Key words: geographic variation; individual specialization; proportional similarity; prey choice

The diets of some octopuses are well described from midden data, however, prey choice by octopuses is poorly understood. At the same time, individual variation in octopus diets is poorly described, although known to exist (*Octopus vulgaris* (Cuvier, 1797): Anderson *et al.* 2008; Mather *et al.* in press; *O. insularis* (Leite *et al.*, 2008): Leite *et al.* 2009).

Many marine animals exhibit such individual variation in diet composition and this is often attributable to the influence of learning on foraging choices (West 1986). For example, for grazing herbivorous sea slugs individual preference was related to grazing history (Jensen 1989) and the high learning costs associated with switching prey (Jensen 1989; Trowbridge 1991). Sea otters also exhibit long-lived, individual variation in diet based on learning (Estes *et al.* 2003) as do seabirds (Woo *et al.* 2008).

Octopuses exhibit problem-solving intelligence (Fiorito 1990; Mather 1995) and spatial memory (Mather 1991b), are capable of short-distance navigation (Alves *et al.* 2008) and of learning both spatial (Boal *et al.* 2000) and foraging tasks (Anderson and Mather 2010). *Octopus cyanea* (Gray, 1849) are speculative tactile foragers (Yarnall 1969; Forsythe and Hanlon 1997). Similar foraging behavior has been documented for other octopuses although typically in less detail (e.g. *Enteroctopus dofleini* (Wülker, 1910): Johnson 1942; Cosgrove 2002; *O. ornatus* (Gould, 1852): Roper and Hochberg 1988; *O. maorum* (Hutton, 1880): Vafiadis 1998; for an exception for *O. vulgaris*: Mather 1991a; Mather and O'Dor 1991) and often primarily through examination of middens (e.g. *E. dofleini*: Hartwick *et al.* 1981; Vincent *et al.* 1998). At the same time, octopuses appear to be time minimizers rather than rate-maximizing foragers (McQuaid 1994; Scheel *et al.* 2007; Leite *et al.* 2009). Curio (1976) suggested that searching predators are unselective of prey because they expend so much energy on search that they will take what results from each search effort. For example, *E. dofleini* learned to forage on a fishing net and used dens nearby (Rigby and Sakurai 2005); but were stationary or hiding > 90% of the time (Scheel and Bisson 2012). For some octopuses, prey selection is also known to vary in relation to octopus body-size (Smith 2003) and among geographic regions (Vincent *et al.* 1998; Ibáñez and Chong 2008).

Octopuses have been variously described as generalists (based on diverse marine benthic taxa appearing in their diet e.g., Bouth *et al.* 2011), and as specialists (because only a few species dominate octopus diet, Mather 1991a; Mather and O'Dor 1991; Vincent *et al.* 1998; Anderson *et al.* 2008). However, increasingly these diets are being described as those of a specializing predator, due to the dietary dominance of few taxa (e.g. *Octopus insularis*: Leite *et al.* 2009; Bouth *et al.* 2011; *O. cyanea*: Mather 2011); because octopuses are individually specialized while generalizing at the population level (*O. vulgaris*: Anderson *et al.* 2008), or because they are known to exhibit size and species selectivity (*Enteroctopus dofleini*: Scheel *et al.* 2007) in their diet. Digestibility and nutritional needs may also lead to individual specializing because although bivalve prey provided higher energy gain rates, *O. rubescens* (Berry, 1953) lipid absorption efficiency was higher on crustacean prey (Onthank and Cowles 2011), *E. dofleini* growth rate was higher on mixed than monotypic diets (Rigby and Sakurai 2004), and cephalopod growth efficiency may be finely tuned to nutrient ratios (Lee 1994).

In this paper, we present new data on the predatory behavior (as represented by diet determined from midden piles) of *Enteroctopus dofleini* and examine the variation in diet and individual specialization at locations from Puget Sound to the Aleutian Islands. The sensitivity of *E. dofleini* behavior to temperature fluctuations is well established (Prescott 1962; Rigby and Sakurai 2004), although geographic variation in this response remains to be worked out. Harley (2011) emphasized the role of marine benthic predator-prey dynamics varying along a geographic gradient in mediating the response of communities to climate change. In this paper we survey *E. dofleini* diet across a wide portion of its geographic range, which data continue to build a foundation for a better understanding of octopus predatory behavior and benthic food-web dynamics.

METHODS

We assessed diet of the giant Pacific octopus, *Enteroctopus dofleini*, from remains of prey individuals found in middens outside octopus dens. Middens were recognized by the presence of an octopus, a den excavated in a characteristic fashion, and/or the presence of distinct marks (drills, bites) indicative of octopus predation on prey remains (Dodge and Scheel 1999). Middens were surveyed by divers (Puget Sound, WA at depths to 10 m; Saanich Inlet, BC at depths to greater than 7 m, Cosgrove 2003–2006; Amchitka and Adak, Aleutian Islands, Alaska at depths to 16 m. Fig. 1), and by beach-walks during low tide series between May and September (Prince William Sound, and Kachemak Bay in Cook Inlet, Alaska. Scheel 2002; Scheel *et al.* 2007). At each den we recorded by species the number of individuals represented in the prey remains. Only the Prince William Sound and Saanich Inlet data were used to examine diet variability over years because (with minor exception) the annual span of data from other regions was two years or less (Table 1). Remains present at a den were only scored as prey if they were fresh, judged by the lack of erosion and encrusting growth (diatoms, algae, spat) on interior surfaces kept clean in life. Thus, midden records represent recent foraging because remains are epiphyte-free for only a few days.

Individual specialization

We examined individual specialization of diet from two measures: the proportional similarity of each midden to the population diet recorded from all middens for each geographic region (which compares the niche breadth of each midden *i* to the niche breadth of the population, Feinsinger *et al.* 1981; Bolnick *et al.* 2002); and Cardona's (1991) niche breadth (B'), which were calculated from occurrence frequency of each prey species *j* = *1....R* in middens:

$$\text{Proportional similarity, } PS_i = \sum^{j} \min(p_{ij}, q_j)$$

$$\text{Cardona's niche breadth, } B' = \left(\sum^{j} f_j - \sigma\right) / (100\, R), \text{ where } f_j = (100\, N_j/N)$$

where p_{ij} is the proportion of prey individuals of species *j* within midden *i*, q_j = proportion of that species across all middens from a sampling region (notation following Bolnick *et al.* 2002), N_j = number of middens containing species *j*, *N* = number of middens, σ = standard deviation of f_j, R = the number of prey species used by the population (notation modified from Cardona 1991). PS_i takes on a minimum value of q_j for individuals specializing entirely on species *j*, and the value 1.0 for individuals leaving prey remains in proportion to the population as a whole. The average of PS_i over a population of middens is a measure of individual specialization within a population. B' has lower values when individuals specialize, and approaches one as all members of a

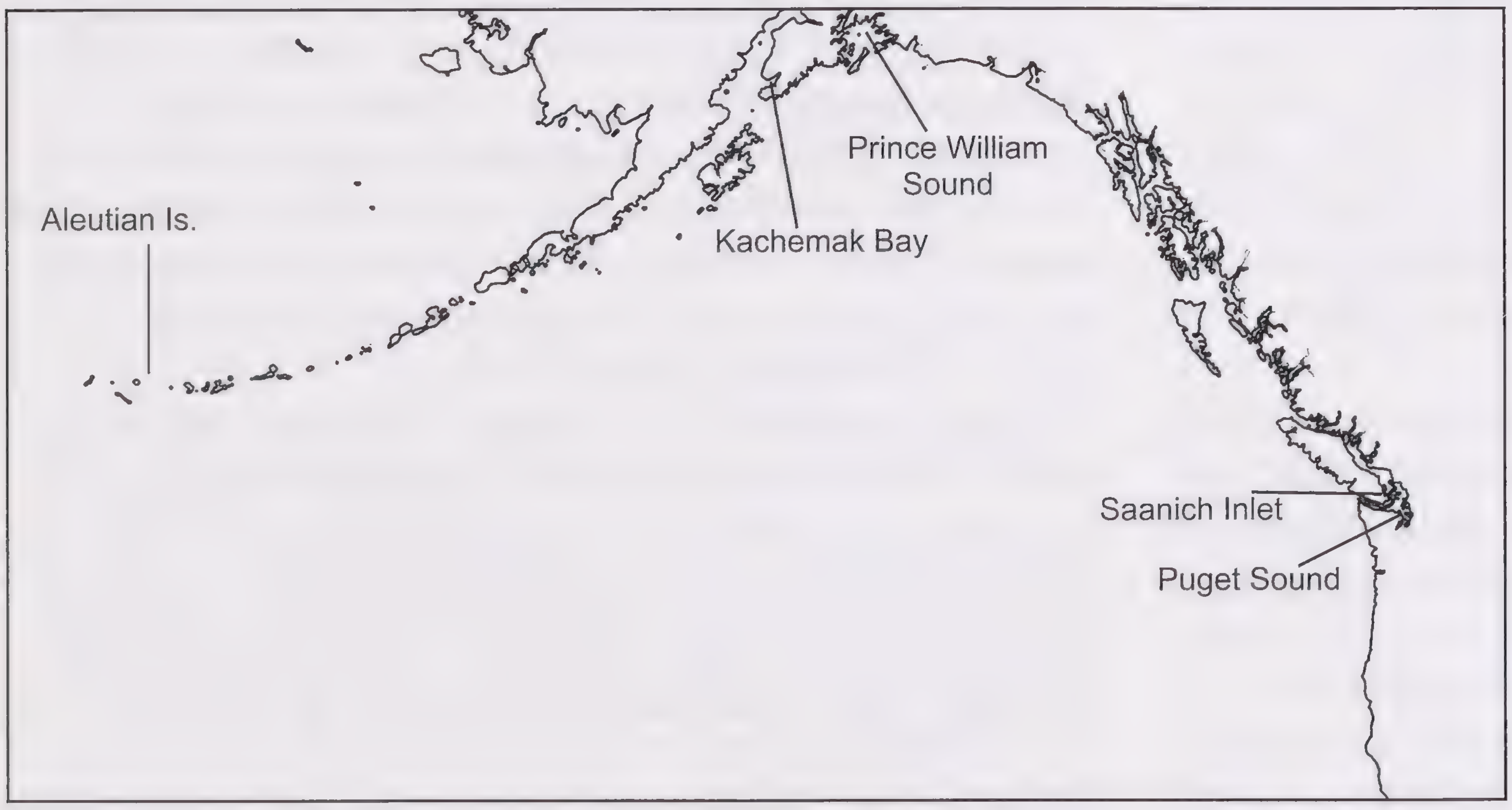

Figure 1. Sampling locations in the north Pacific. 'Aleutian Islands' indicates the approximate location within the chain where sampling was conducted at Amchitka and Adak Islands.

Table 1. Minimum number of prey individuals, *N*, represented in middens (proportion of total prey items) (percent of middens where present) at dens of giant Pacific octopuses by geographic region (see text for details). Bold indicates dietary dominance (= $N_1/\sum N_j$, N_j ranked s.t. j = 1 for the most common species, see Methods for details). Cardona's (1991) niche breadth is calculated for each region from proportions of middens containing each species.

Taxonomic name	Puget Sound	Saanich Inlet	Prince William Sound	Cook Inlet	Amchitka & Adak	Total
CRUSTACEA: *Cancer productus* J.W. Randall, 1840	**389 (.45)** (81)	**476 (.81)** (99)	617 (.20) (60)	2 (.01) (5)		1484 (.30)
CRUSTACEA: *Cancer oregonensis* (Dana, 1852)	1 (.00) (2)		**766 (.25)** (65)	148 (.42) (70)	2 (.03) (20)	917 (.19)
CRUSTACEA: *Telmessus cheiragonus* (Tilesius, 1812)	3 (.00) (4)	16 (.03) (13)	528 (.17) (51)	**156 (.44)** (91)		703 (.14)
CRUSTACEA: *Pugettia gracilis* Dana, 1851	2 (.00) (2)		373 (.12) (37)	21 (.06) (25)	5 (.08) (40)	401 (.08)
BIVALVIA: *Pododesmus macrochisma* (Deshayes, 1839)	137 (.16) (19)		80 (.03) (11)	1 (.00) (2)	**20 (.32)** (70)	238 (.05)
CRUSTACEA: *Lophopanopeus bellus* (Stimpson, 1860)			207 (.07) (30)	2 (.01) (5)		209 (.04)
BIVALVIA: *Clinocardium nuttallii* (Conrad, 1837)	107 (.12) (42)		53 (.02) (6)	1 (.00) (2)		161 (.03)
BIVALVIA: *Saxidomus gigantea* (Deshayes, 1839)	76 (.09) (35)		54 (.02) (9)	3 (.01) (5)		133 (.03)
CRUSTACEA: *Pugettia producta* (J.W. Randall, 1840)	10 (.01) (15)	79 (.14) (28)	1 (.00) ()			90 (.02)
BIVALVIA: *Protothaca staminea* (Conrad, 1837)	10 (.01) (12)		74 (.02) (11)	5 (.01) (5)		89 (.02)
BIVALVIA: *Macoma* sp.[a] Leach, 1819		2 (.00) (1)	59 (.02) (8)	4 (.01) (5)		65 (.01)
BIVALVIA: *Chlamys hastata* (Sowerby, 1842)	38 (.04) (4)		5 (.00) (1)			43 (.01)
CRUSTACEA: *Cancer magister* Dana, 1852	37 (.04) (25)		2 (.00) ()			39 (.01)
CRUSTACEA: *Hapalogaster mertensii* Brandt, 1850			31 (.01) (6)	4 (.01) (9)		35 (.01)
GASTROPODA: *Euspira lewisii* (Gould, 1847)	7 (.01) (10)	2 (.00) (1)	20 (.01) (4)	1 (.00) (2)		30 (.01)
CRUSTACEA: *Cancer gracilis* Dana, 1852	25 (.03) (17)					25 (.01)
CRUSTACEA: *Cryptolithodes sitchensis* Brandt, 1853			24 (.01) (3)			24 (.00)
BIVALVIA: *Modiolus modiolus* (Linnaeus, 1758)			2 (.00) ()		20 (.32) (40)	22 (.00)
BIVALVIA: Unidentified			20 (.01) (3)			20 (.00)
BIVALVIA: *Humilaria kennerleyi* (Reeve, 1863)	17 (.02) (10)		2 (.00) ()			19 (.00)
POLYPLACOPHORA: *Tonicella lineata* (W. Wood, 1815)			17 (.01) (4)			17 (.00)
BIVALVIA: *Mytilus* sp.[b] Linnaeus, 1758	3 (.00) (4)		11 (.00) (2)	1 (.00) (2)		15 (.00)
BIVALVIA: *Gari californica* (Conrad, 1849)	1 (.00) (2)		14 (.00) (3)			15 (.00)
ECHINOIDEA: *Strongylocentrotus droebachiensis* (O.F. Müller, 1776)			14 (.00) (3)			14 (.00)
CRUSTACEA: *Hemigrapsus* sp.[c] Dana, 1851		4 (.01) (2)	7 (.00) ()	1 (.00) (2)		12 (.00)
BIVALVIA: *Tresus capax* (Gould, 1850)	1 (.00) (2)		3 (.00) (1)	2 (.01) (2)		6 (.00)
TELEOSTEI: various		2 (.00) (2)	2 (.00) ()			4 (.00)
GASTROPODA: *Nucella lima* (Gmelin, 1791)					4 (.06) (10)	4 (.00)
GASTROPODA: *Nucella lamellosa* (Gmelin, 1791)	1 (.00) (2)		3 (.00) (1)			4 (.00)
CRUSTACEA: *Scyra acutifrons* Dana, 1851		3 (.01)				3 (.00)
BIVALVIA: *Serripes groenlandicus* (Mohr, 1786)					3 (.05) (10)	3 (.00)
CRUSTACEA: unidentified lithoid crab		1 (.00)	1 (.00) ()			2 (.00)
BIVALVIA: *Clinocardium ciliatum* (Fabricius, 1780)					2 (.03) (20)	2 (.00)
BIVALVIA: *Crassostrea gigas* (Thunberg, 1793)	1 (.00) (2)					1 (.00)
BIVALVIA: *Entodesma saxicola* (Baird, 1863)	1 (.00) (2)					1 (.00)
POLYPLACOPHORA: *Cryptochiton stelleri* (Middendorff, 1847)	1 (.00) (2)					1 (.00)
Individuals of other taxa[d]		(3)	47 (.02) (11)		6 (.10) (50)	53 (.01)

Table 1. (Continued)

Taxonomic name	Puget Sound	Saanich Inlet	Prince William Sound	Cook Inlet	Amchitka & Adak	Total
Total prey items represented	868	585	3037	352	62	4904
[d]Number of other taxa found	-	-	24	-	5	30
Total taxa found (species richness)	21	9	52	15	12	69
Average Proportional Similarity, PS	0.45	0.77	0.40	0.48	0.34	
Cardona's niche breadth (B')	(.13)	(.14)	(.11)	(.14)	(.30)	
Total number of den visits	52	87	440	44	10	633
Years of sample	1996 (n = 1) 2005 (n = 1) 2009–2010	2002–2006	1997–1998 2001–2011	2010	2011	

[a] *Macoma* was identified as *M.* sp. in Puget Sound and as *M. inquinata* (Deshayes, 1855) in PWS.

[b] *Mytilus* sp. was identified as *M. trossulus* Gould, 1850 in Puget Sound, and as *M. edulis* Linnaeus, 1758 in PWS.

[c] *Hemigrapsus* was recorded as *H.* sp. in Puget Sound and as *H. oregonensis* (Dana, 1851) or *H.* sp. in PWS.

population have identical diets. Finally, we used R (species richness) and the Berger-Parker diversity index (indicating dominance, d = maximum of q_j, Magurran 1988) as additional measures of regional octopus dietary breadth.

We tested whether each midden represented a random sample from the regional population of all prey represented in middens using the likelihood ratio,

$$\lambda_i = \Pi_j \left(q_j / p_{ij}\right)^{n_{ij}}$$

where Π_j denotes the serial product over species j, and n_{ij} is the number of prey items of species j in midden i. The quantity $-2 \ln (\lambda_i)$ has an approximately chi-square distribution with (R-1) degrees of freedom (Bolnick et al. 2002). As this test was run for each of the 633 middens (Table 1), we used a more conservative $p < 0.001$ to identify significantly specialized middens.

RESULTS

Diets were compared from a sample of middens collected in the years 1996–2011 at octopus dens in intertidal and sub-tidal sites (Table 1; prey items per midden: mode = 4, range n_i = 1 to 58). Over all areas (Fig. 1), octopus middens contained remains of 69 taxa, but 80% of all prey came from just five crab species and the bivalve *Pododesmus macrochisma* (Deshayes, 1839). Another 10% of all prey came from an additional three bivalve species and the crab *Pugettia producta*, so that across all areas 90% of prey items came from only ten species.

Regional diets

Diet as represented by middens differed among sampled regions (Table 1, Fig. 1). In Saanich Inlet, where dietary species richness was low (R = 9 species) and dietary dominance high (d = 81% of prey items from one species), *Cancer productus* was the most common prey in the middens. *Cancer productus* similarly dominated middens in Puget Sound, however, richness and dominance were both moderately high. In south-central Alaska, five crab species, including both *C. productus* and *C. oregonensis* were common in middens in Prince William Sound (PWS), where the highest richness and lowest dominance were found (Table 1). Three of these crabs were also prevalent in Cook Inlet middens, although richness and dominance were moderate. *Telmessus cheiragonus* and *C. oregonensis* were abundant in middens in both these south-central regions, where middens were predominantly of intertidal dens. Both richness and dominance were low in middens from Amchitka and Adak Islands because these middens were dominated by two bivalve species, and relatively few other species were represented.

Further, middens in Puget Sound were larger than in the other regions (Puget Sound, $\bar{x} = 16.6$ prey/midden; other regions, $\bar{x} = 6.9$. Chi-squared test, $df = 16$, $\chi^2 = 59.6$, $p < .001$). The dominant species in Puget Sound and Saanich Inlet (*Cancer productus*, to carapace width 20 cm) is a larger crab than the crab species that replace it in Alaska middens (*Telmessus cheiragonus* to 10 cm, *C. oregonensis* to 4.3 cm, *Pugettia gracilis* to 3.2 cm). The other common prey in Puget Sound middens were large bivalve species (*Pododesmus macrochisma*, *Clinocardium nuttallii* (Conrad, 1837), and *Saxidomus gigantea* (Deshayes, 1839)) which comprised smaller portions of the diet in most other areas (Table 1), such that middens in Puget Sound especially represent more food than those found in British Columbia (fewer prey) or in south-central Alaska (fewer prey and smaller species of prey).

The size, richness and composition of middens varied across the years 2002–2006. A typical midden early during this period contained more prey individuals and had higher species richness than middens later, in both PWS and BC, although the timing of the decline was not the same in both locations (Fig. 2). Smaller middens thus became more frequent. Composition of the diet also changed across years. In both Prince William Sound and Saanich Inlet, the proportion of the population diet comprised of *Cancer productus* increased in the period 2003 to 2005 from the 2002 level; while those of *Pugettia gracilis* and *Telmessus cheiragonus* decreased in the same period (Fig. 3). In the Prince William Sound sample, the midden representation of other small crabs such as *Cancer oregonensis* also varied across years. *Cancer oregonensis* was more abundant in the middens of Prince William Sound octopuses in 2003 and 2005 relative to

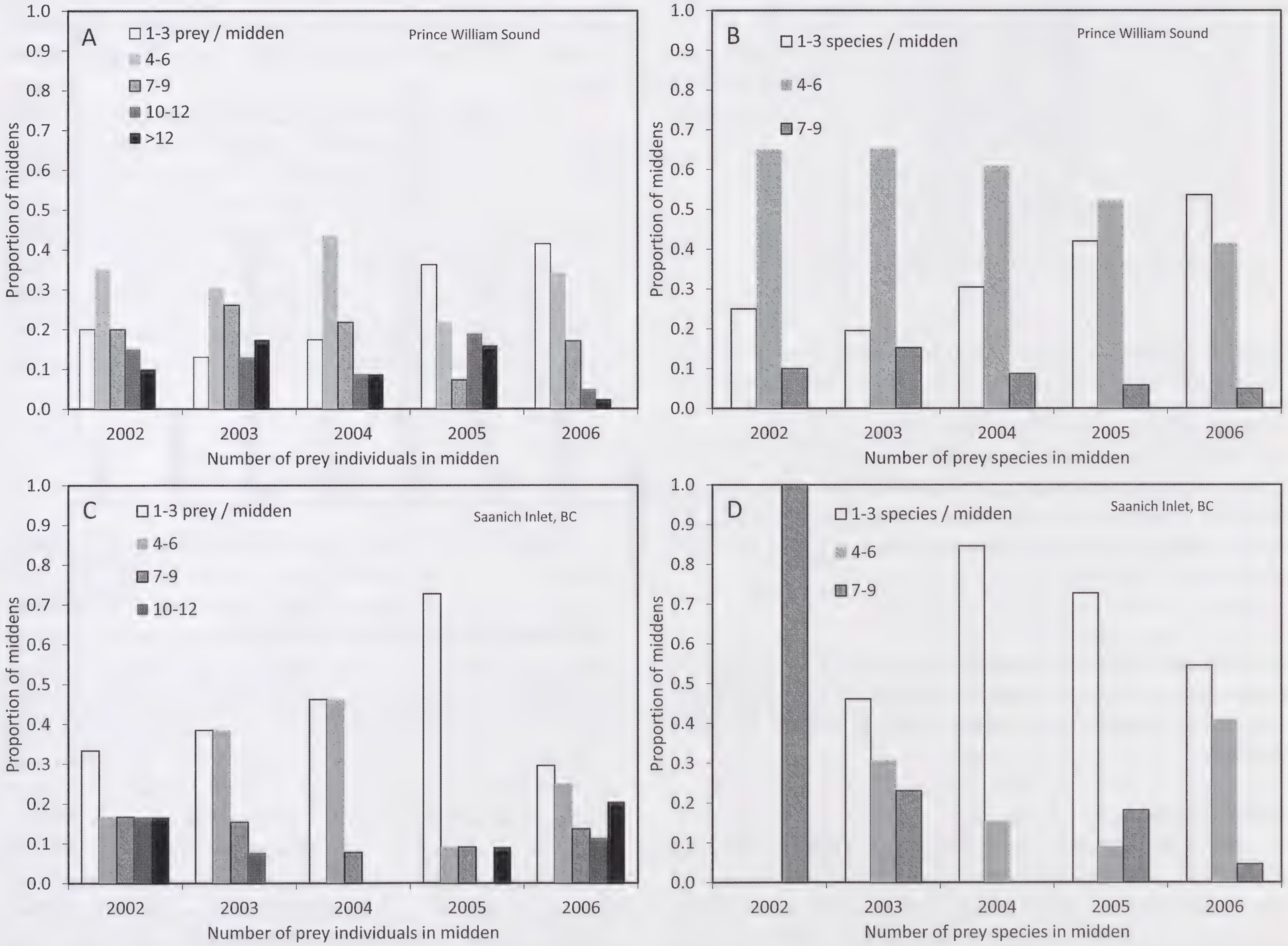

Figure 2. Midden size (proportion of middens by number of prey individuals, **A**, **C**) and richness (proportion of middens by number of species, **B**, **D**) left by giant Pacific octopuses in Prince William Sound and Saanich Inlet, BC, by year of sampling.

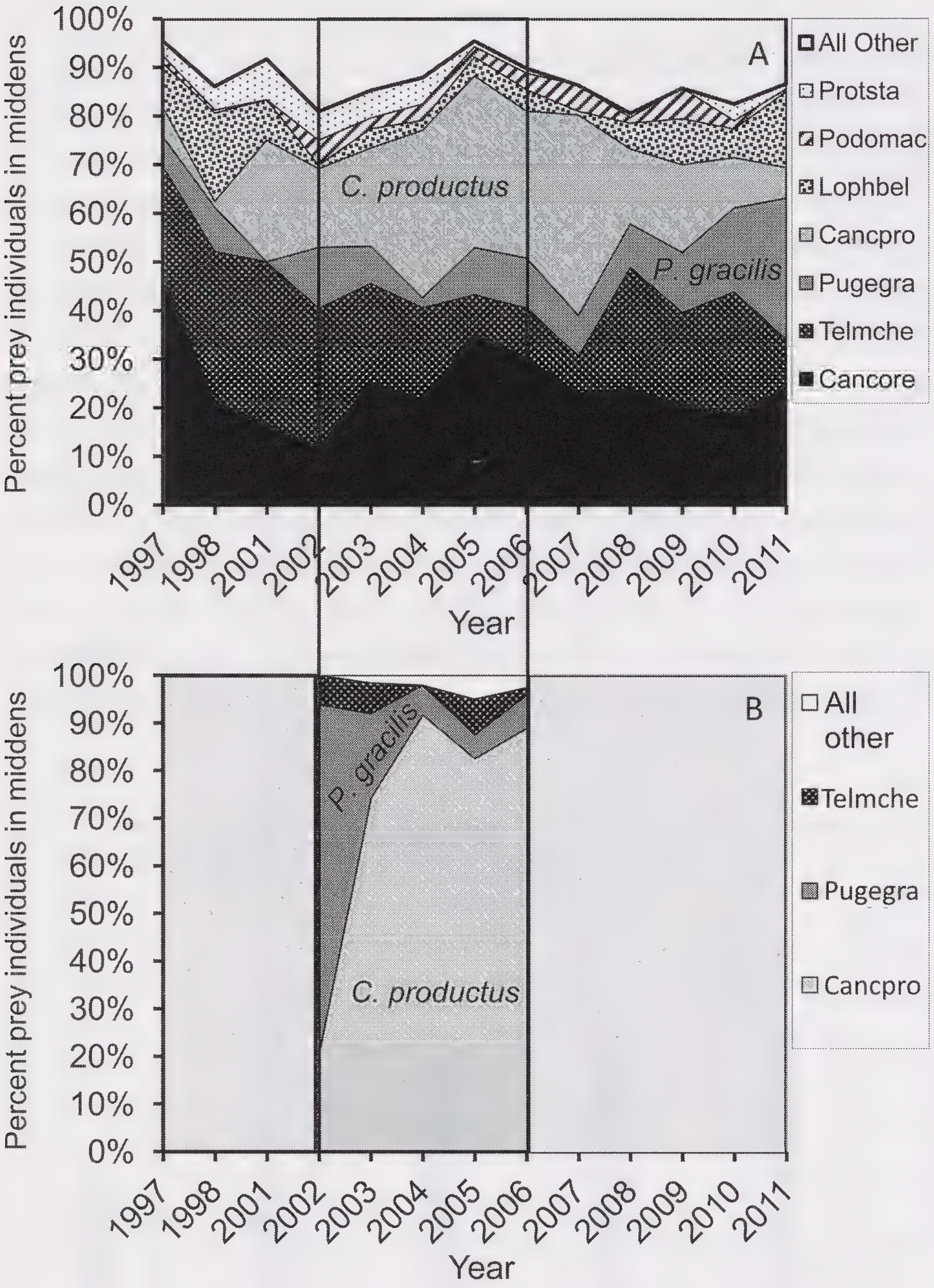

Figure 3. Species composition of prey individuals found in middens left by giant Pacific octopuses in Prince William Sound (**A**, remains of 3037 prey individuals represented in 440 middens) and Saanich Inlet, BC (**B**, remains of 585 prey individuals represented in 90 middens) by year of sampling. Species abbreviations consist of the first four letters of the genus name and first three letters of the specific name (i.e. *Prototheca staminea* becomes Protsta, see Table 1 for full species names). Data were unavailable for Saanich Inlet except for years shown.

2002. Because prey species differ in size, fewer individuals in the midden may not represent lower caloric intake, as a larger species (*C. productus*) was substituted for smaller (*Pugettia gracilis*).

Individual diets

Most individual octopus middens did not significantly differ from the regional population of midden items over all years (Figs. 4–5, Puget Sound $N = 10$ of 52 middens differed: df = 21, $\lambda_i > \chi^2_{21,\, p < .0001} = 46.8$; Saanich Inlet $N = 2$ of 87 middens: df = 9, $\lambda_i > \chi^2_{9,\, p < .0001} = 27.9$; PWS NS $N = 440$ middens: df = 54, $\lambda_i < \chi^2_{54,\, p < .0001} = 86.7$; Cook Inlet NS $N = 44$: df = 15, $\lambda_i < \chi^2_{15,\, p < .0001} = 37.7$; Aleutians NS $N = 10$: df = 12, $\lambda_i < \chi^2_{12,\, p < .0001} = 32.9$). Overall the frequency distribution of midden proportional similarities varied little by regions (Fig. 4), except for Saanich Inlet, BC, where the mode was .81 to .9. Midden richness was low and dietary dominance high in Saanich Inlet (Table 1); but these middens differed little from the population sample. The population prey was dominated by a single species (Table 1, *Cancer productus*), most middens contained only that prey species (50 of $N = 87$ middens), and nearly all middens (99%) contained at least one *C. productus*. The only two instances of specialization in Saanich Inlet were on the crab *Pugettia producta*. In contrast, for Puget Sound, 10 of 52 middens (19%) differed significantly, and individuals specialized on the bivalves *Clinocardium nuttallii*, *Pododesmus macrochisma*, *Saxidomus gigantea* ($N = 2$ each), *Humilaria kennerleyi* (Reeve, 1863), *Chlamys hastata* (G. B. Sowerby II, 1842) ($N = 1$ each) or on the crabs *Cancer magister* or *Cancer productus* ($N = 1$ each). In all cases of significant specialization, the midden contained remains of at least 12 prey items, typically over twenty (Fig. 5).

Patterns of specialization with items per midden varied across regions. In Puget Sound (and also Cook Inlet) proportional similarities averaged about 0.5 (Table 1) but there was no relationship to items per midden (Fig. 5). This was different in Saanich Inlet, where proportional similarities averaged nearly 0.8, although there was still no relationship to items per midden. However, in Prince William Sound and in the Aleutians, proportional similarities on average were only slightly lower than in Puget Sound, but increased with items per midden.

DISCUSSION

Human Aleut hunter gatherers subsisting on intertidal marine resources in the Aleutian Islands, Alaska have been characterized as super-generalists, consuming > 25% of available species, more than any other animal foraging in the intertidal (Travis 2012). That study, based on data from middens left by humans over a 5000-year period, found that Aleuts ate 50 different species. In this report, we document comparable species richness in the diet of *Enteroctopus dofleini* from samples spanning just 17 years, including 52 intertidal species where diet was most diverse (Prince William Sound) and 69 species over all regions (Table 1). Thus, *E. dofleini* appears under certain conditions also to act as a super-generalist forager. *E. dofleini* were specialists within a generalist population only where middens and dominant prey were both large (Puget Sound), while the population specialized only where large prey species dominated in middens of smaller modal size (Saanich Inlet). In contrast,

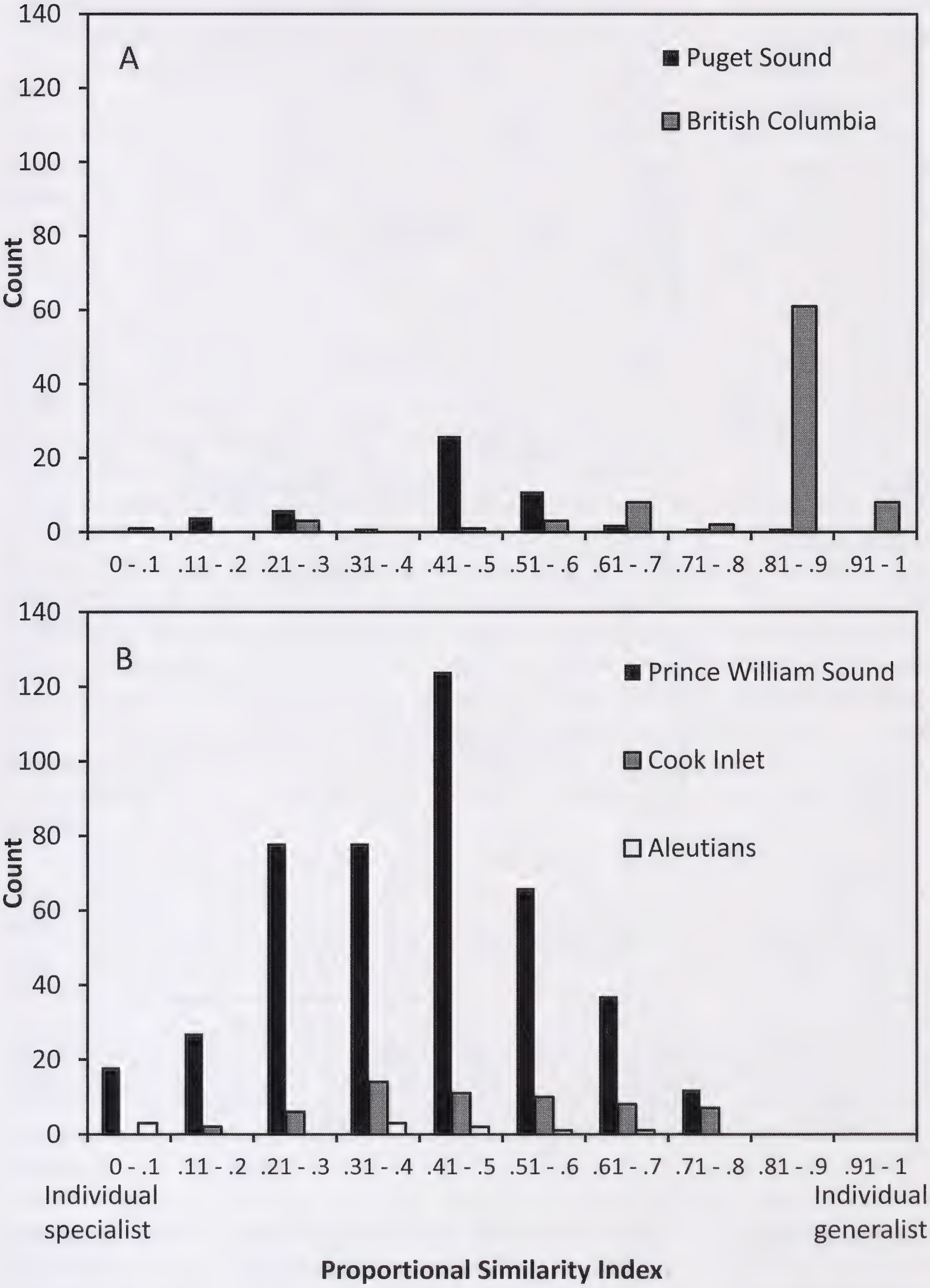

Figure 4. Frequency distribution of proportional similarity of middens left by giant Pacific octopuses to the population midden sample for Puget Sound and Saanich Inlet (**A**) and three areas in Alaska (**B**).

E. dofleini were generalists when middens and prey were both smallest (Prince William Sound).

Several factors may contribute to these regional differences in dietary specialization, but they can be considered as factors that affect how well middens represent diet (Mather 1991a), and factors affecting octopus foraging (including differences in prey availability). First, middens are dynamic (Ambrose 1983, Mather 1991a) as midden remains are removed by both biotic agents (e.g. hermit crabs) and erosion of items from the midden by burying or by currents. Hermit crabs affect the residence-time of snail shells in middens (Ambrose 1983), but gastropods were only a minor component of middens in our study. Exposure of middens to waves and currents also does not appear to explain differences in midden richness, composition and specialization. For example, the relative abundance of bivalves (less likely to bury or be swept away) to crustacean exoskeletons (more likely to be removed, Mather 1991a) was highest in the Aleutians and Puget Sound, and lowest in Kachemak Bay and Saanich Inlet (Table 1). Current exposure may be high in the Aleutians but summer wave exposure was not (because the samples were collected at depth); Kachemak Bay has high wave exposure (long fetch and intertidal sampling); while Saanich Inlet and Puget Sound are both highly protected waters. Thus any hypothesis of differential erosion of the middens as a function of local wave energy appears unable to explain the differences we observed.

Are differences in midden composition and specialization consistent with explanations of octopus behavior other than changes in diet selection behavior? For example, octopus den residency time may have differed among regions. The accumulation of midden items over longer periods (or alternatively by multiple individual octopuses each using the den for shorter periods) could result in more items and greater species richness per midden relative to a midden left by one octopus over a short time period. However, we included only fresh prey remains (see methods) in our sample, and thus limited the variation across regions in midden age. *Enteroctopus dofleini* forages near its den (Mather et al. 1985, Scheel unpublished data) every two to three days or more frequently (Scheel and Bisson 2012), and thus, fresh prey remains from middens generally will include only remains from the one or two most recent foraging bouts (plus any prey recently taken near the den). Variation in den residency time or foraging frequency thus

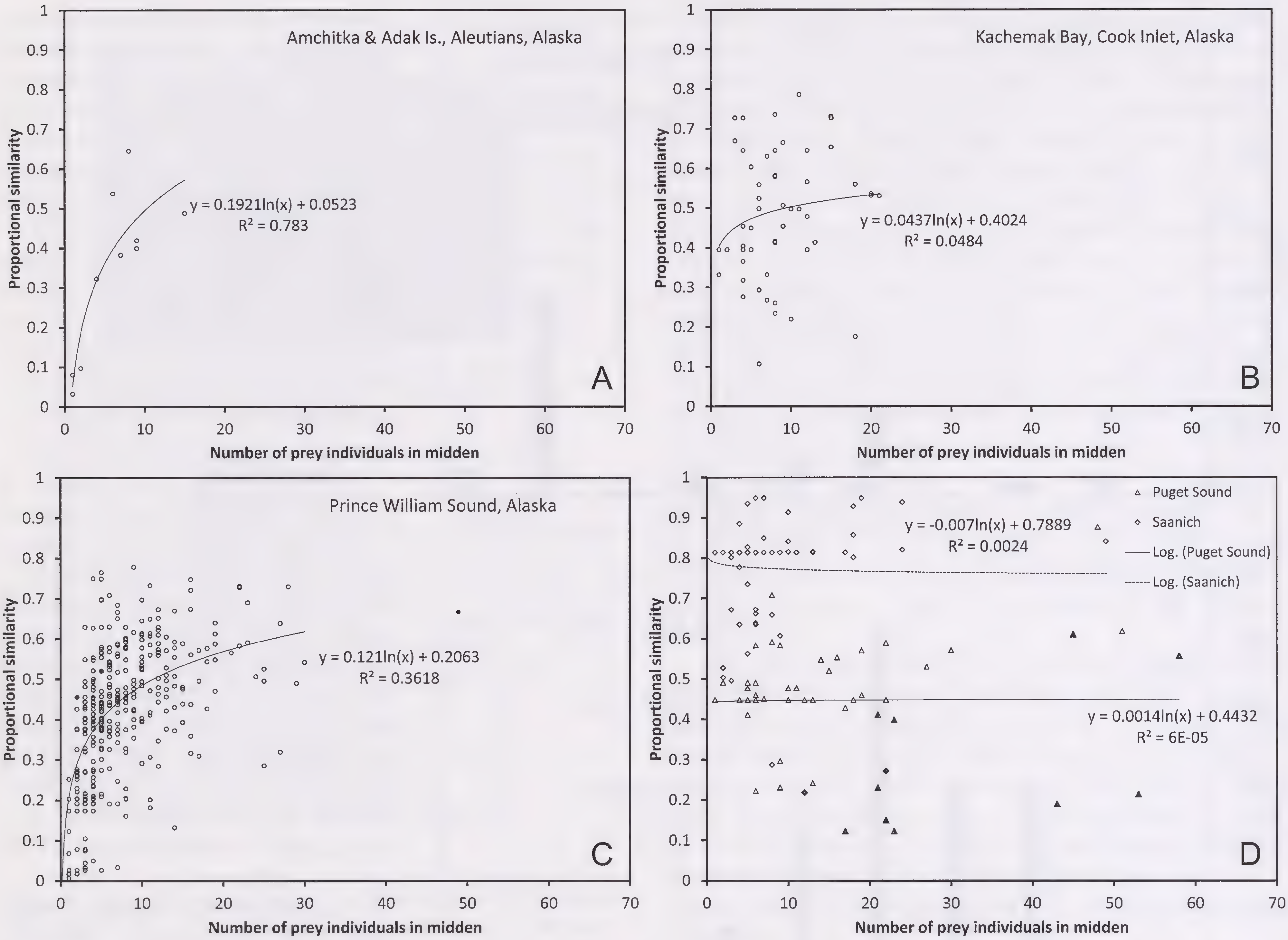

Figure 5. The proportional similarity of middens (each point represents one midden) left by giant Pacific octopuses to the population midden sample by items per midden in each sampling area. Large filled symbols (**D**, Puget Sound, Saanich Inlet) indicate midden contents representing statistically significant specialization in diet (see methods). Lines (and equations) indicate natural logarithmic fit to data.

appears unable to explain regional variation in the specialization of midden contents.

In contrast, our results are consistent with the hypothesis that octopus diet selection behavior was different among sampling regions: when large preferred prey were abundant in middens, foraging *Enteroctopus dofleini* all specialized in the same way (perhaps as rate-maximizing foragers). When large preferred prey were not abundant in middens, individuals appeared to take prey as encountered, leading to a relationship between PS_i and the number of items per midden (Alaska, Fig. 5). Thus, we observed that specialization and food content per midden both were reduced from Puget Sound north and west across the range. The distinction between specialization (proportion of individual diet comprised of one species) and similarity (of one diet to the population, PS_i) is important (see Mather *et al.* in press). In Saanich Inlet where *C. productus* occurred in 99% of middens, average PS_i was high but in another sense nearly every midden was specialized on this single species.

Is it possible to say that midden contents reflect octopus foraging responses to habitat quality or habitat richness? Intertidal species richness is high in south central Alaska and peaks just below the depth where intertidal octopus dens are most abundant (Konar *et al.* 2009, 2010, Pohle *et al.* 2010). Scheel *et al.* (2007) found no *Enteroctopus dofleini* population response to densities of live prey in the habitat, but did find that individual midden contents reflected changes in prey abundance. *Enteroctopus dofleini* foraging outcomes also responded to changing prey populations, as average crab size available in the environment declined for some species during a time in which average prey size in the middens increased, indicating increased selectivity by octopuses (Scheel *et al.* 2007). Similarly, Ambrose (1984) found that midden contents of *Octopus bimaculatus* reflected aspects of both prey preference and prey availability. Further, different octopus species behave differently and the relationship of these differences to prey availability is poorly known. *Octopus rubescens* fed predominantly on the most widely available prey in its habitat (Anderson *et al.* 1999), but *O. cyanea* selectively chose just a few crab species despite wide availability of alternate prey (Mather 2011). *Octopus tehuelchus* (D'Orbigny, 1834) in intertidal habitats exhibited considerable prey selectivity possibly connected to chemical deterrents in the prey (Iribarne *et al.* 1991). *Enteroctopus dofleini* also exhibits species selectivity in prey choice (Vincent *et al.* 1998). Thus, midden contents are unlikely to mirror prey availability exactly, but instead will be influenced by availability, octopus prey preferences (e.g. for larger prey, Scheel *et al.* 2007), and variation in foraging effort (foraging habitat selection, Scheel and Bisson 2012; foraging time, etc.). Clearly dietary specialization may occur where octopuses forage most successfully both at the population level (Table 1, R varies from 9 to 52) and the individual level (Fig. 5: 10% of Puget Sound middens were specialized).

In both Saanich Inlet, BC and Prince William Sound, AK during the years 2002 to 2006, *Cancer productus* comprised an increasing proportion of octopus diet (Fig. 3), and the proportion of middens containing only a few prey individuals increased, as did that of middens containing only a few species (Fig. 2). *Cancer productus* is a large crab, and the proportion of middens containing a smaller species, *Pugettia gracilis*, was less in both areas when *C. productus* dominated middens. Because *C. productus* first appeared on live prey surveys at Prince William Sound sites in 2004 (Scheel *et al.* 2007), and their abundance increased in this time period (Scheel, unpublished data), this suggests three things. First, *C. productus* is a preferred prey whose representation in the diet scales with abundance. Second because *C. productus* increased in the diet in both areas, a coastal-wide *C. productus* recruitment event may have preceded these years. Third, an abundance of larger prey may shelter smaller prey such as *P. gracilis* from octopus predation, a conclusion consistent with selectivity of octopuses for larger individuals (Scheel *et al.* 2007); and with predictions of optimal foraging theory that a greater range of prey types will be eaten the lower the overall abundance of food (Schoener 1971).

The specialization in Puget Sound of 10% of middens notwithstanding, the dominant pattern is not one of individual specialization within a generalist population. Similarly, Mather *et al.* (in press) found populations of mixed specialists and generalists across five populations of three octopus species (*Octopus vulgaris*, *O. cyanea*, *Enteroctopus dofleini*). Octopus growth rate may be higher on mixed species diets (Rigby and Sakurai 2004) and most octopuses appeared not to specialize (Fig. 5), even where middens indicated the most food consumed (Puget Sound). At the same time, *E. dofleini* diet may be highly dominated by a single prey or be extraordinarily diverse (Table 1) but in each case individuals in the population all behaved similarly: a specialized population in Saanich Inlet; and a generalized population without specializing individuals in Alaska. This pattern is apparent in the relation of items per midden (sample drawn from the prey population) to midden proportional similarity: in a generalizing population, individual generalization increased towards an asymptote as items per midden increased (Fig. 5, Alaska). In a specialized population, there was no relationship, as the population diet was dominated by a single species and again all individuals resemble the population as a whole (Saanich Inlet). Thus diet specialization, while variable, appears to be a function of foraging success as indicated by prey species' maximum size and number of consumed individuals.

In contrast, Cardona's index of niche breadth (B', Table 1) revealed little of differences between regions in specialization as it did not differentiate clearly between Alaska samples (generalists) and those further south (population or some individual specialization), except in the case of the Aleutian octopuses. The Aleutian B' was more than twice that of other populations and diet in these samples was evenly dominated by just two species; one of which occurred in 70% of all middens. These two species were both large bivalves and this is the only sample dominated by bivalves rather than crustaceans. Onthank and Cowles (2011) found that *Octopus rubescens* have lower lipid absorption efficiencies on bivalve than crustacean prey. It is notable in this context that these bivalves were both large and abundant at the Aleutian sampling sites compared to other Alaska sites (Scheel pers. observation). The hypothesis that Aleutian octopuses rely on bivalves because this prey is sufficiently superabundant to offset lower digestive efficiency could not be assessed in this study.

Prey availability affects many aspects of predator ecology, but especially diet and foraging behavior. Adaptive foragers may select diet to maximize gain rates (MacArthur and Pianka 1966), minimize risk (Stephens and Charnov 1982), or to reduce missed-opportunity-costs (Schoener 1971; Brown 1988) as well as possibly due to nutrient or processing constraints (e.g. Onthank and Cowles 2011). Our results indicate that where middens contained fewer, smaller prey items, *Enteroctopus dofleini* was more generalized in diet, presumably a result of including smaller prey

(Scheel *et al.* 2007; as predicted by rate-maximization theory, Stephens and Krebs 1986) and spending more time foraging, each of which alter the predator's functional response to prey (Holling 1959). It has also been suggested that octopuses follow a win-switch strategy (Mather 2008) in which localized prey depletion favors selection of new habitat, perhaps by changing movement strategies (Scheel and Bisson 2012). Finally, octopus growth rates, and thus time to maturation, vary with food supply (Hartwick *et al.* 1981; Robinson and Hartwick 1986), so that population response to changes in prey availability may occur, although these may not be detectable against a background of variable recruitment (Garstang 1900; Rees and Lumby 1954; Scheel *et al.* 2007).

Biological trait variation, including variation in diet selection, occurs between species and may even drive speciation among eco-types (e.g. killer whales, Baird *et al.* 1992; Pilot *et al.* 2010). Such variation also occurs among individuals as a result of individual developmental and learning histories (Darwin 1859; Baldwin 1896) and contributes to personality differences (Mather and Anderson 1993; Sinn *et al.* 2010). In these analyses we can see variation among individuals within and also among populations. For example, African lions exhibit variation in specialization of hunting roles, which occurs in some populations (Stander 1992) but not others (Scheel and Packer 1991); and individual lions may exhibit specialization of diet even within a cooperatively hunting group (Yeakel *et al.* 2009). Like these examples, variation in individual specialization of diet among northeastern Pacific populations of *Enteroctopus dofleini* lies between that of the species and the individual, and may be related to local environments and individuals' learned differences. Unfortunately, few data about prey abundances and micro-habitats, or data about octopus growth rates, were available for these populations and we were unable to examine the role of environmental differences in accounting for population differences. Data on individual histories of foraging octopuses will be even more difficult to obtain.

Declines in prey abundance have led to a higher reliance on small prey and greater dietary species richness among Chugach Alutiiq natives in south central Alaska foraging on the same prey types and in the same habitats as these octopuses (Salomon *et al.* 2007). Similarly, Caddy and Rodhouse (1998) and Piatkowski *et al.* (2001) suggest that cephalopods are increasing in the world oceans because of depletion of their predators and competitors; and a rise in generalist species has been reported in many other contexts (Clavel *et al.* 2010). The flexible response of octopuses to be either specialists or generalists indicates that ecosystems may also lose specialists if foraging success declines as habitat quality or richness declines. Clavel *et al.* (2010) categorize species as specialists primarily according to morphological parameters, and by this measure, coleoid cephalopods may be the ultimate generalists using suckers, beak, radula, and venom to capture and consume a wide variety of prey whenever a generalist diet maximizes aspects of fitness.

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Sex bias in interspecific copulation between the hermaphroditic freshwater snails, *Physa acuta* and *P. pomilia* (Physidae)

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Abstract. Previously-published mate-choice experiments have uncovered significant prezygotic reproductive isolation between South Carolina populations of *Physa acuta* (Draparnaud, 1805) and *P. pomilia* (Conrad, 1834). Here we report the results of similar tests returning no evidence of such barriers between an Alabama population of *P. pomilia* and three separate populations of *P. acuta*, two from Alabama and one from South Carolina. However, heterogametic copulations did demonstrate both a significant sex bias and a significant size bias. The smaller *P. acuta* was more likely to copulate in the male role and hence less exposed to any potential fitness decrements due to the reduced viability and sterility of the hybrids produced. Our observations on defeated mating attempts suggested that *P. acuta* may be more aggressive than Alabama *P. pomilia* in mating contests (its smaller size perhaps contributing to greater agility) and hence more likely to prevail in the male role. The absence of a sex bias in our previously-published mate-choice observations involving South Carolina *P. pomilia* and *P. acuta* may be attributable to our use of size-matched snails, which equalized the agility of the prospective copulants, thus perhaps prolonging the mating contests, and ultimately lessening the likelihood that any mating took place at all.

Key words: Sexual isolation, reinforcement, mate-choice tests, gender conflict, pulmonate gastropods.

Speciation in animal populations is typically thought to begin with the evolution of reproductive incompatibility in allopatry, after which prezygotic reproductive isolating mechanisms may evolve, possibly reinforced by natural selection (Coyne and Orr 2004). Thus in well-studied systems it is not unusual to discover populations of biological species demonstrating incomplete prezygotic reproductive barriers, some pairs having evolved sensory or behavioral cues to reduce the frequency of interspecific copulation, and others copulating indiscriminately (e.g., *Drosophila* Gleason and Ritchie 1998, finches Grant and Grant 2002).

Physa acuta (Draparnaud, 1805) and *P. pomilia* (Conrad, 1834) are closely-related freshwater pulmonate gastropod species that separate into two monophyletic groups based on sequence data with ranges broadly overlapping throughout the southern United States (Wethington and Lydeard 2007). They differ in minor details of shell morphology, penial anatomy, and life history (Wethington *et al.* 2009). *Physa acuta* demonstrates a "weedy" life history strategy, populations reaching maximum density in more unpredictable or disturbed habitats, such as farm ponds or impoundments. *Physa pomilia* populations are more typical of stable habitats, such as the quiet backwaters of rivers or permanent streams. Hybrids between *P. acuta* and *P. pomilia* are sub-vital and sterile (Dillon *et al.* 2007). But although the mate-choice experiments of Dillon and colleagues (2007) returned evidence of significant prezygotic reproductive barriers between South Carolina populations of *P. acuta* and *P. pomilia*, no such evidence was returned by the experiments of Dillon *et al.* (2011) matching South Carolina *P. pomilia* with a population of *P. acuta* from Pennsylvania. This suggested to Dillon and colleagues that the behavioral differences leading to assortative mating among the South Carolina populations might represent adaptations, being reinforced by natural selection.

Here we extend this line of research to Alabama, performing mate-choice tests to include an Alabama population of *Physa pomilia* and two Alabama populations of *P. acuta*, as well as our South Carolina *P. acuta*. We also expand our observations to gather data on the polarity of interspecific copulation, the relative sizes of the copulants involved, and the frequency at which copulations may be entirely defeated by rejective behaviors.

Physid snails are simultaneously hermaphroditic at adulthood, female function being added to male function around week 7–8 in laboratory culture (Wethington and Dillon 1993). They are preferential outcrossers, suffering a significant reduction in fitness if compelled to self-fertilize in isolation (Wethington and Dillon 1997, Jarne *et al.* 2000). Copulation is not reciprocal. Pairs of snails typically engage in contests to determine which individual will initially assume the male role, after which swapping may occur (Facon *et al.* 2008). By comparing snails of differing reproductive histories, Wethington and Dillon (1996) adduced evidence that such contests are attributable both to (conventional) sexual conflict and to a phenomenon they termed "gender conflict" between simultaneous hermaphrodites vying to assume a male role.

In addition to prior reproductive history, the relative size of prospective copulants can influence the gender initially assumed by copulating freshwater pulmonate snails, the smaller partner more often mating in the male role (Ohbayashi-Hodoki *et al.* 2004, Norton *et al.* 2008). DeWitt (1991, 1996) correlated such observations with the size advantage model for simultaneous hermaphrodites (Charnov 1982, Angeloni *et al.* 2002). He documented four behaviors that *Physa* may display in their attempts to discourage copulation as a female: shell shaking, increasing the distance from the gonopore, covering the gonopore with the shell, and biting the partner's preputium.

All of our mate-choice experiments published to date have been specifically designed to test for reproductive isolation among populations of *Physa*, and hence have involved size-matched snails in an explicit effort to factor out effects such as those described by DeWitt. Few of the previous reports have included data on the directionality of any heterogametic copulations that may have been observed. Here we add these layers of observation in an effort to cast additional light on the nature of prezygotic reproductive barriers between *P. acuta* and *P. pomilia*.

METHODS AND METHODS

We collected approximately 50 adult snails from each of four natural populations, the identities of all of which were confirmed by dissection. *Physa pomilia* was sampled from the type locality of the species: Randons Creek, 12 km S of Claiborne, Monroe County, Alabama (31.4387°N, 87.5445°W). This population was previously designated "P" by Dillon *et al.* (2007), and "alpom" in the mtDNA sequence analysis of Wethington and Lydeard (2007). Our population "*acuta*-A" was sampled from Lake Palmer on the University of Alabama campus (33.2151°N, 87.5467°W), and our population "*acuta*-T" was sampled from Cypress Pond, southeast of Tuscaloosa, Alabama (33.0659°N, 87.6373°W). Our control population "*acuta*-C" was sampled from the main pond at Charles Towne Landing State Park, west of the Ashley River in Charleston, South Carolina (32.8062°N, 79.9862°W). This population has previously been designated "Ctl" by Dillon and Wethington (1995), "C" by Dillon *et al.* (2002, 2005), "A" by Dillon *et al.* (2004, 2007) and "*acuta*-C" by Dillon *et al.* (2011). Twenty 16S and CO1 mtDNA sequences from this population were published by Wethington *et al.* (2009). The habitat has been described by Dillon and Dutra-Clark (1992).

Wild-collected snails were returned to the laboratory, isolated for a minimum of seven days in 10 oz. (210 ml) clear polyethylene drinking cups of aerated, filtered pond water with Petri dish covers, and fed a commercial *Spirulina*-based flake fish food, finely ground. All cultures took place at approximately 23° C in a 12:12 light cycle. Snails demonstrating the ability to lay viable eggs served as experimental animals for our mate-choice tests.

We performed three separate mate-choice experiments to assess prezygotic reproductive isolation: *pomilia* x *acuta*-C, *pomilia* x *acuta*-T, and *pomilia* x *acuta*-A. Each experiment comprised two trials involving five adult snails per population. Since statistics to measure sexual isolation in simultaneous hermaphrodites have conventionally been calculated on the basis of the first successful copulation initiated by each snail in the male role (to avoid double-counting), the maximum number of mate-choice observations in each experiment was 20. Note that our experimental design yields a slightly unbalanced expectation, each snail being presented with five heterogametic partners but only four homogametic.

For each trial, the two groups of five snails were measured with calipers, painted with a dot of fingernail polish (either pink or silver) and numbered with an ultra-fine Sharpie pen. They were then placed together in a glass container 19 cm in diameter and 6.5 cm high, elevated on a glass plate platform. Approximately 750 ml of filtered pond water was added to the container, bringing the depth to approximately 2.7 cm. We then monitored mating activity over a period of two hours, recording every copulation attempt. When a successful copulation was observed, defined as the complete insertion of the penis of one partner into the mantle cavity of a second, both snails were identified and their gender roles recorded. Snails were not removed or disturbed during the two-hour observation period, however, and were often observed to engage in multiple mating interactions. Thus we were also able to record the total number of defeated mating attempts in each experiment--those mating interactions that did not ultimately culminate in copulation by either partner.

We tested the hypothesis of prezygotic reproductive barriers between *Physa pomilia* and each of the three *P. acuta* populations using the Index of Pair Sexual Isolation (IPSI), a statistic designed to remain unbiased should two populations demonstrate differing mating propensities (Perez-Figueroa *et al.* 2005, Carvajal-Rodríguez and Rolán-Alvarez 2006). We then tested the (one-tailed) hypothesis that smaller snails might be more likely to copulate in the male role with a chi-square goodness-of-fit test on the entire data set, combined over all three experiments. Focusing finally on the subset of copulations between heterogametic partners, we used a goodness-of-fit chi square statistic to test for evidence of bias in the gender assumed by the two species and a matched-pair t statistic to test the (one-tailed) hypothesis that the snails copulating in the male role tended to be smaller.

RESULTS

Table 1 shows our primary data sorted by experiment and the relative size of the copulants. The average size of the 30 *P. pomilia* ultimately involved in our experiment was 8.26 mm (range 6.15–11.15), as compared to 7.99 mm (range 6.00–9.50) for the 10 *acuta*-C, 7.83 mm (range 6.00–10.10) for the 10 *acuta*-A, and 5.47 mm (range 4.40–6.25) for the 10 *acuta*-T. Only 7 of the 20 snails tested in the *pomilia* x *acuta*-C experiment had copulated in the male role when the two hour time limit expired, 12 (of 20) in the *pomilia* x *acuta*-T experiment, and 15 (of 20) in the *pomilia* x *acuta*-A experiment. The 14 copulations totaled for *pomilia* x *acuta*-A in Table 1 exclude one homogametic pairing identically matched in size.

Table 1 reflects no evidence of assortative mating by population or by the relative size of the copulants. The IPSI index in the *pomilia* x *acuta*-T experiment was 0.26 (p = 0.184) and in the *pomilia* x *acuta*-A experiment IPSI = 0.20 ($p = 0.221$). The *pomilia* x *acuta*-C experiment returned more heterogametic copulations observed than homogametic copulations. Combining the three independent tests of the same hypothesis using the Fisher method (Sokal and Rohlf 1995: 794) yielded a nonsignificant result ($\chi^2 = 6.70$, 6 *df*). Summed over all three experiments, a test of the hypothesis that males tended to be the smaller of the two partners yielded a goodness-of-fit $\chi^2 = 2.46$, also not significant.

Focusing on the subset of 16 heterogametic copulations, however, Table 2 does demonstrate a significant bias toward the male role in *Physa acuta* ($\chi^2 = 4.00$, $p < 0.05$). And the average size of the male copulants in this subset (mean = 7.11 mm, range 4.90–9.50) was significantly smaller than the snails ultimately copulating as female (mean = 7.80 mm, range 5.65–9.65), our paired t-test returning t = 1.89 (one-tailed, $p = 0.039$). It would appear that smaller *acuta* tend to prevail over larger *pomilia* in interspecific gender conflicts.

Table 1. The distribution of initial copulations observed in mate-choice tests involving Alabama *Physa pomilia* and three populations of *P. acuta*.

	Male smaller	Male larger	Total
pomilia x *acuta*-C			
Homogametic	1	1	2
Heterogametic	4	1	5
pomilia x *acuta*-T			
Homogametic	4	3	7
Heterogametic	4	1	5
pomilia x *acuta*-A			
Homogametic	4	4	8
Heterogametic	4	2	6
Totals	21	12	33

Table 2. The subset of copulations shown in Table 1 involving heterogametic partners.

	Male smaller	Male larger	Total
Male *acuta*	9	3	12
Male *pomilia*	2	2	4
Totals	11	5	16

We observed all of the rejective behaviors described by DeWitt (1991) during the course of these experiments, as well a variety of others. Often snails mounted as females were observed to crawl out of the water in an apparent attempt to dislodge an eager partner. Biting (or perhaps head-butting) was applied to all the exposed surfaces of prospective suitors, including the foot as well as the preputium. "Dancing," a term coined by us, was applied to situations where a pair of prospective copulants pressed their feet together and twisted about in a vigorous fashion. Such "dancing" typically seemed to result when a pair of snails nearly matched in their size encountered each other head-on, and vied to assume the upper (male) position.

The number of entirely defeated mating attempts, culminating in no copulation by either partner, exceeded the tally of successfully copulating partners in all three experiments. Table 3 shows that individual *Physa acuta* sampled from all three populations were significantly more rejective than *P. pomilia*. While only three *pomilia* x *pomilia* homogametic pairings ultimately failed to yield a successful copulation over all 12 hours of observation combined, 24 pairs of *acuta* ultimately defeated each other (Fisher's $p = 0.0012$) . This figure is comparable to the 23 *pomilia* x *acuta* heterogametic pairings that did not ultimately culminate in copulation by either partner (Fisher's $p = 0.074$).

DISCUSSION

Our previously-published observations on hybrid fitness led us to expect that some prezygotic reproductive isolation

Table 3. Defeated mating attempts (not culminating in copulation by either partner) observed in mate-choice tests involving *Physa pomilia* and three populations of *P. acuta*.

Experiment	*pomilia* x *pomilia*	*pomilia* x *acuta*	*acuta* x *acuta*
pomilia x *acuta*-C	1	4	3
pomilia x *acuta*-T	2	14	10
pomilia x *acuta*-A	0	5	11
Totals	3	23	24

might be evident between Alabama populations of *Physa pomilia* and *P. acuta*, not perhaps apparent in interactions between Alabama *pomilia* and our standard population of *P. acuta* from South Carolina (Dillon *et al.* 2007, 2011). The mate-choice experiments we report here do not, however, reflect any evidence of assortative mating between any pair of populations tested. The behavioral mechanisms that seem to have evolved to lessen the frequency of copulation between South Carolina populations of *P. acuta* and *P. pomilia* did not appear effective in our samples from Alabama.

Although our experiments are patterned closely after the "mate-choice tests" that have seen broad application to measure sexual isolation between populations of a great variety of dioecious animals, each of our observations is not a "choice" in any sense, but rather the outcome of a contest (Wethington and Dillon 1996). The victor obtains the male role. The data in Table 3 demonstrate that Alabama *pomilia* mate more often with *acuta* than with other *pomilia*. But the data in Table 2 suggest that ultimately, it is *Physa acuta* (smaller, and perhaps more agile) that tends to prevail as male more often.

The observation that males tend to be eager and indiscriminant in their mating, while females tend to be passive and choosy, has been a familiar one since the days of Darwin. Bateman (1948) suggested that the asymmetry in the energetic investments required in the production of egg and sperm should lead to greater sexual selection among males than females, a theory that was extended to hermaphrodites by Charnov (1979). The phenomena of egg trading (Crowley *et al.* 2007) and sperm trading (Michiels *et al.* 2003, Anthes *et al.* 2006) have been viewed as adaptations to resolve the (male-female) sexual conflicts in simultaneous hermaphrodites, and direct contests such as those observable in freshwater pulmonates typically ascribed to (male-male) gender conflict (Facon *et al.* 2007, 2008).

In dioecious animals, one well-documented basis for female choosiness is the suite of mating cues offered by courting males (e.g., appearances, behaviors, chemicals), with selection acting to refine the female's ability to recognize mates yielding maximum fitness for her offspring (Milam 2010). This is the theoretical basis of mate choice tests (Bateson 1983). Although such selective forces also certainly apply to simultaneous hermaphrodites (Pelissie *et al.* 2012), it seems possible that in the 16 cases documented in Table 2, eagerness for victory in a (male-male) gender conflict has led to defeat in a (male-female) sexual conflict.

In direct contrast to the results reported here, the mate-choice experiments involving South Carolina populations we reported in 2007 did return evidence of reproductive isolation between *pomilia* and *acuta* while reflecting no sex bias in heterogametic pairings. Only 11 of the 41 copulations logged by Dillon and colleagues (2007) were heterogametic, five with *pomilia* as male and six with *acuta* as male, not statistically different from 1:1. But the snails we selected for our earlier experiments were size-matched as closely as possible, in an explicit effort to factor out size-related differences in aggression or agility. In equalizing the body sizes of all prospective copulants, we almost certainly extended the contests among heterogametic partners, ultimately lessening the likelihood that copulation took place in either direction at all.

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Population sex ratios of pleurocerid snails (*Leptoxis* spp.): Variability and relationships with environmental contaminants and conditions

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Abstract. Anthropogenic introduction of steroidal estrogens and synthetic estrogenic compounds to aquatic environments has increased interest in assessment of the endocrine-disrupting effects of these compounds on aquatic organisms, including mollusks. For gonochoristic freshwater snails in the family Pleuroceridae, little is known about the spatial and temporal variation in population sex ratios or potential relationships between sex ratios and exposure to estrogenic compounds. Population sex ratios of *Leptoxis* (Rafinesque, 1819) spp., were evaluated within the Shenandoah River watershed (Virginia, U.S.A.), where agricultural operations are a source of estrogenic compounds to streams, and in four other river basins in Virginia. Proportions of females varied among streams within the Shenandoah River watershed, with overall mean proportions from four sampling periods ranging from 0.46–0.87. Sex ratios were consistently female-biased at nine of fifteen stream sampling sites. There was little within-site variation across generations of snails or when the same generation was examined in two different seasons. Proportions of females were not directly related to in-stream concentrations of estrogenic compounds or watershed densities of agricultural operations, but were negatively related to mean summer temperature at the sampling sites. Population sex ratios of *Leptoxis* spp. were female-biased at two of six sites in the Shenandoah River and one of five sites outside the basin. At the river sampling sites, proportions of females were only related to summer-specific conductivity. Overall, results suggest that site-specific factors can affect population sex ratios of *Leptoxis* spp. However, until more is known regarding mechanisms of sex determination and sexual differentiation in gastropods, population sex ratios should not be used as indicators of potential biological effects of estrogenic compounds.

Key words: gastropods, female-biased, estrogenic compounds, spatial variation

The presence of steroidal estrogens and synthetic estrogenic compounds in aquatic environments from anthropogenic sources, such as wastewater treatment plant (WWTP) effluent and manure from animal feeding operations (AFOs), has raised concern regarding the effects of these contaminants on wildlife (Jobling *et al.* 1998, Petrovic *et al.* 2002, Hanselman *et al.* 2003, Johnson *et al.* 2006). Their effects on male fish are well studied, and include inappropriate production of egg-yolk precursor proteins (vitellogenin) and the presence of oocytes in the testes (intersex) (Jobling *et al.* 1998, Jobling *et al.* 2003, Hahlbeck *et al.* 2004, Fenske *et al.* 2005, Lange *et al.* 2009). Laboratory exposures of fish to estrogens and estrogenic compounds during early life stages have resulted in complete feminization of genetic males and female-biased sex ratios (Hahlbeck *et al.* 2004, Fenske *et al.* 2005, Lange *et al.* 2009, Yonkos *et al.* 2010). In wild fish populations, changes from balanced to female-biased sex ratios have been observed along gradients of influence from WWTP effluent discharges (Woodling *et al.* 2006, Vajda *et al.* 2008, Duffy *et al.* 2009) and gradients of watershed agricultural intensity (Jeffries *et al.* 2008). Thus, anthropogenic introduction of estrogens and estrogenic compounds to aquatic environments may affect fish populations through disruption of sexual differentiation.

Similar to fish, sexual development in mollusks appears to be susceptible to alteration by estrogens and estrogenic compounds. Exposures of freshwater and marine mussels to estrogens and estrogenic WWTP effluents during early stages of gametogenesis increased production of vitellogenin- and vitellin-like proteins (Gagne *et al.* 2001, Quinn *et al.* 2004) and expression of vitellogenin mRNA (Ciocan *et al.* 2010) in males and females. Aqueous exposures of undifferentiated oyster larvae to nonylphenol induced hermaphroditism and female-biased sex ratios in the adult population, and these population characteristics were not present in unexposed organisms (Nice *et al.* 2003). Studies of potential effects of estrogens and estrogenic compounds on wild mollusk populations are limited. In a Canadian river, population sex ratios of freshwater mussels changed relative to locations of WWTP discharges; sex ratios were male-biased upstream of the discharges and female-biased downstream (Gagne *et al.* 2011). Although these results suggest that population sex ratios of freshwater mollusks may be viable indicators of exposure to estrogens or estrogenic compounds, there is a lack of understanding of the mechanism for mollusks' apparent susceptibility to endocrine disruption by these compounds. The functional role of estrogens in mollusks remains unclear (Ketata *et al.* 2008, Matthiessen 2008) and molluscan estrogen receptors do not appear to actually bind estrogen (Thornton *et al.* 2003, Kajiwara *et al.* 2006, Keay *et al.* 2006, Bannister *et al.* 2007). In addition, the spatial and temporal variability of population sex ratios of many species is unknown.

Measures of spatial and temporal variability of population sex ratios are necessary in order to discern potential effects of environmental conditions, or contaminants like estrogens and estrogenic compounds, on this population characteristic. In gonochoristic species with genetic sex determination, expected population sex ratios are balanced (i.e. 1:1 or proportions of males or females = 0.5) (Fisher 1930, Bull 1983). Freshwater snails in the family Pleuroceridae (Superorder Caenogastropoda: Order Sorbeoconcha: Superfamily Cerithioidea) are gonochoristic with sexual reproduction and limited studies of other freshwater caenogastropods suggest that sex is genetically determined (reviewed by Dillon 2000, Yusa 2007). Population sex ratios ranging from balanced to female-biased have been observed for pleurocerid snails (reviewed by Dillon 2000, see also Aldridge 1982, Hendrix 1986, Miller-Way and Way 1989). However, samples were generally not collected at multiple sites over multiple seasons.

The Shenandoah River (Virginia, U.S.A.) and its tributaries support large populations of the pleurocerid snail *Leptoxis carinata* (Bruguière, 1792), with population densities as high as 3,000 snails/m^2 (Orth *et al.* 2009). Smallmouth bass populations in the Shenandoah River have a high proportion of intersex males relative to other basins, suggesting a possible biological effect of exposure to estrogens and estrogenic compounds (Blazer *et al.* 2007). Discharges from WWTPs and over 1200 AFOs are potential sources of these compounds in the Shenandoah River watershed, and significant relationships between watershed densities of AFOs and estrogenicity in Shenandoah River tributaries were recently documented (Ciparis *et al.* 2012). The specific objectives of this study were to: 1) document variation in sex ratios of *L. carinata* in Shenandoah River tributaries, 2) compare these sex ratios with those of *Leptoxis* spp. in the Shenandoah River and other rivers in Virginia, and 3) assess relationships between population sex ratios and land use, environmental variables, and other population characteristics. The working hypothesis was that sex ratios in *Leptoxis* spp. populations would vary from balanced to female-biased, and that proportions of females in these populations would be related to landscape sources and measured concentrations of estrogens and estrogenic compounds (as total estrogenicity). However, because of limited knowledge of the mechanisms of sex determination and differentiation in pleurocerid snails, relationships between sex ratios and other land use and environmental variables, as well as snail population density and degree of parasitic infection, were also explored.

MATERIALS AND METHODS

Study sites

Study sites in the Shenandoah River watershed were selected to represent a gradient of influence from AFOs and municipal WWTPs. Briefly, locations of AFOs (poultry, dairy, and beef) and WWTPs were obtained from Virginia state agencies. For individual Shenandoah River tributaries, total densities of AFOs and WWTP presence were quantified within delineated 12-digit, 6th level Hydrologic Unit Code (HUC 6) subwatersheds (40–160 km^2 in size). Twenty-five sites, located near outlets of the delineated HUC 6 subwatersheds, were sampled during a preliminary study in 2007. Fifteen sites in 12 Shenandoah River tributaries had relatively large populations of *Leptoxis carinata* in stable riffles and were selected for the current study (Fig. 1). Three of the tributaries drained multiple subwatersheds, and an upstream (US) sampling site was located in the primary subwatershed as well as a downstream (DS) site draining multiple subwatersheds. This created three sets of upstream-downstream pairs. Across the 15 sampling sites, watershed densities of AFOs ranged from 0–4.2 operations/1,000 acres, and six sites had permanent WWTP discharges located upstream. Land cover percentages of forest, pasture/hay, cultivated crops, and developed land were quantified for each watershed as detailed in Ciparis *et al.* (2012). All land use characteristics were quantified using ArcGis 9.3 (ESRI, Redlands, California).

Sampling sites in Virginia rivers were part of a larger study of geographic variation in fish health (Orth *et al.* 2009). Four sites were located in the North Fork of the Shenandoah River, with an upstream site located near Cootes Store (A), followed by downstream sites near Mount Jackson (B), Woodstock (C), and Strasburg (D) (Fig. 1). Two sites were located in the South Fork of the Shenandoah River, an upstream site in the North River above its confluence with the South River (E) and a downstream site in the South Fork Shenandoah River near Luray (F) (Fig. 1). Five locations outside of the Shenandoah River watershed were selected: the Rappahannock River near Richardsville (G), the Cowpasture River at Walton Tract (James River basin; H), the James River upstream of Buchanan (I), the New River near Draper (J), and the North Fork Holston River upstream of Saltville (K) (Fig. 1). Land use characteristics were not quantified upstream of river sites due to the large watershed sizes and the resulting mixture of land uses present at the watershed scale.

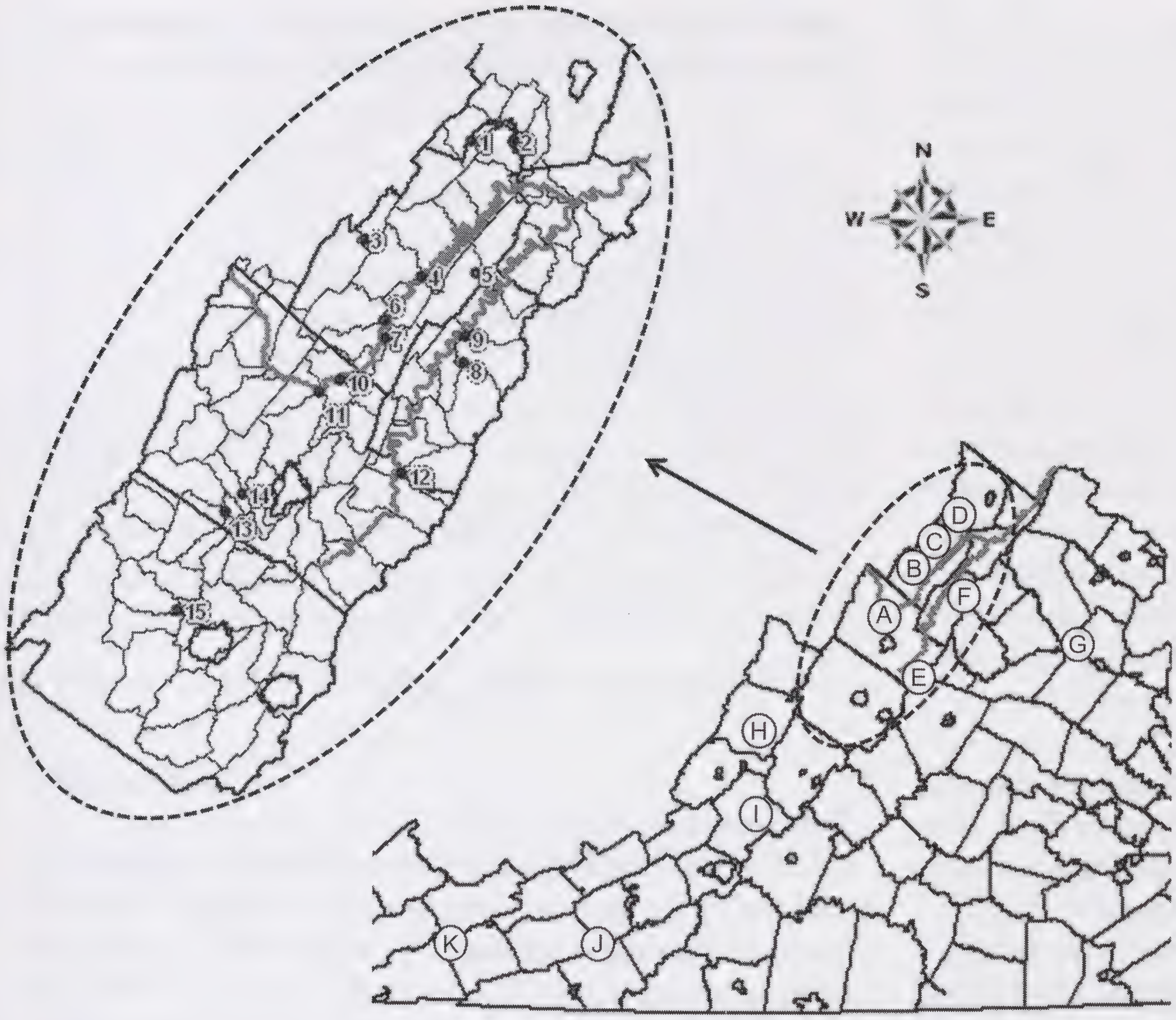

Figure 1. Locations of sampling sites in rivers (letters) relative to counties in Virginia and locations of sampling sites in tributaries (numbers) within the Shenandoah River watershed (enlargement). Within the Shenandoah River watershed, the two forks of the river are indicated by the grey line and the 12-digit hydrologic unit code subwatersheds are outlined.

Snail sampling

Leptoxis carinata was the only species of pleurocerid snail encountered at all Shenandoah River and James River basin sites and *Leptoxis dilatata* (Conrad, 1835) was the only species encountered at the New River site. At the Rappahannock River site, *Elimia virginica* (Say, 1817) and *L. carinata* were present, but only *L. carinata* was sampled due to dominance in riffle areas. At the Holston River site, *Pleurocera uncialis* (Haldeman, 1841) and *Leptoxis subglobulosa* (Say, 1825) were present, but only *L. subglobulosa* was sampled due to dominance in riffle areas. All species identifications were verified against reference collections at the Smithsonian Institution National Museum of Natural History (Washington, DC).

Previous studies of *Leptoxis carinata* and *L. dilatata* have shown that these species are semelparous biennials; 2-year old snails lay eggs between late spring and mid-summer and die by late summer (Aldridge 1982, Hendrix 1986, Miller-Way and Way 1989). Only adult (> 1 yr old, > 7 mm shell length) snails were included in sex ratio calculations because sexes are not morphologically distinct until the second summer of life (Aldridge 1982, Hendrix 1986, Miller-Way and Way 1989, verified in Ciparis 2011). At Shenandoah River tributary sites, population sex ratios were determined for three generations of *L. carinata.* The 2006 generation was sampled 14–24 May 2008, the 2007 generation was sampled 5–15 October 2008 and 21 May–19 June 2009, and the 2008 generation was sampled 2–23 October 2009. The 2007 generation was sampled twice to assess sex ratio variability. To further assess seasonal variability, additional sampling of adult snails in 2008 and 2009 generations was conducted over 16 months (12 sampling dates) at two Shenandoah tributary sites: one with equal sex ratios (Briery Branch) and one with female-biased sex ratios (Long Meadow Run). Population sex ratios were determined for only two generations of *Leptoxis* spp. at river sites. The 2006 generation was sampled 2–16 June 2008 and the 2007 generation was sampled 19–27 October 2008.

At each study site, quadrat sampling at randomly selected locations in one riffle was performed. A Surber sampler (0.0929 m^2) was used at tributary sites, and a portable invertebrate box sampler (PIBS; 0.1 m^2) was used at river sites. Prior to sampling, it was determined that a minimum of 96 sexed snails were required to allow 95% confidence in the accuracy of the estimated proportion of females within ± 0.1 if the expected proportion of females was 0.5 (Ott and Longnecker 2001). During 2008, four replicates were collected at both tributary and river sites. During 2009, eight replicates were collected at tributary sites in order to increase the sample size. For the study of seasonal variation in sex ratios at two tributary sites, adult snails were collected within four quadrats (Surber). After collection, juvenile and adult snails in each replicate sample were counted. Adults were retained and transported to the laboratory in site water.

Adult snails were narcotized in 1% $MgCl_2$ in site water for 8–12 hours. In 2008, snails were not measured but in 2009, all snails from each replicate were measured with a digital caliper and placed into 1-mm size classes. Size classes were pooled for determination of sex ratios, except during the intensive sampling of the two tributary sites, where sex ratios for each size class were calculated separately during each collection period. Snails were removed from their shells and sexed using a dissecting microscope. Females were determined by presence of an egg-laying groove on the right side of the foot and developing oocytes in the ovary. Male pleurocerid snails do not have a penis; males were determined by absence of the egg-laying groove, the presence of a fleshy pad in its place, and examination of the testes. The gut/gonad complex and rectum of each snail was examined for developing cercariae of digenetic trematodes. Confirmation of infection was performed by examination of excised cercariae under a compound microscope (10x–40x). Infected snails were counted for determination of parasitic infection rates, but only uninfected snails were used to determine sex ratios since infection precludes gonad development and full development of the egg-laying groove. Five apparent hermaphrodites (egg-laying groove and testes) were observed during the study and were not included in sex ratio calculations.

Environmental measurements

Environmental data were collected during spring sampling periods, during mid-summer (tributaries: 8–14 Aug. 2008 and 10–11 Aug. 2009, rivers: 6–24 Aug. 2008), and during late winter (10–15 March 2009; tributary sites only). Midsummer was selected instead of fall in order to ensure that data could be collected during near-baseflow conditions and relatively stable water temperatures. Temperature (°C) and specific conductivity (μS/cm) were measured at each site using a calibrated YSI 30 Salinity/Conductivity/Temperature meter (YSI Incorporated, Yellow Springs, Ohio). Water samples were analyzed for nutrient concentrations (μg/L), including total dissolved inorganic N (DIN) and PO_4-P, and estrogenic activity (as ng/L 17β-estradiol equivalents) as detailed in Ciparis *et al.* (2012). Mean seasonal temperatures and specific conductivity were used in data analysis; different years were pooled (tributary sites) due to the potentially high variability of point measurements, but spring and summer were separated due to large seasonal differences (paired t-test, $p < 0.0001$ for both parameters). Mean concentrations of nutrients and estrogenic activity were used in data analysis because season/flow conditions did not have a significant effect on concentrations of these analytes at either tributary sites (Ciparis *et al.* 2012) or river sites (paired t-tests, $p \geq 0.073$). Periphyton was collected during mid-summer from four rocks per riffle using a wire brush, bar-clamp sampler (9.62 cm^2), and deionized water. Following methods in Steinman *et al.* (2006), samples were filtered, chlorophyll a was extracted and quantified spectrophotometrically, and ash-free dry mass (AFDM) was determined. Mean chlorophyll a (μg/cm^2) and AFDM (mg/cm^2) from the four replicates were pooled across years for tributary sites. The two measurements were linearly related, so only chlorophyll a was used in data analysis. Summary statistics for all environmental measurements are shown in Table 1.

Table 1. Ranges of environmental variables for sampling sites in Shenandoah River tributaries and five rivers in Virginia.

	Tributary sites	River sites
Spring temp. (° C)[a]	13.0–19.9	15.8–30.1
Summer temp. (° C)[a]	19.2–24.5	22.4–27.6
Spring sp. conductivity (μS/cm)[a]	83.8–625	102–414
Summer sp. conductivity (μS/cm)[a]	171–618	154–514
17β-estradiol equivalents (ng/L)[b]	0.19–3.4	0.16–1.2
PO_4-P (μg/L)[b]	2.91–471	2.31–95.9
DIN (μg/L)[b]	36.62–6,589	62.82–2,637
Chlorophyll a (μg/cm^2)[c]	2.0–28	0.35–15

[a] Measured in May and August; tributaries = mean of 2008 and 2009, rivers = 2008 only

[b] Tributaries = mean of five sampling periods (May and August 2008, March, May and August 2009); rivers = mean of two sampling periods (May and August 2008)

[c] Measured in August; tributaries = mean of 2008 and 2009, rivers = 2008 only

Data analysis

The total numbers of males and females collected in all replicates were used to calculate sex ratios for each site during each sampling period. Sex ratios are presented as proportions of females (number of females/total number of sexed snails). Deviations from expected proportions (0.5 for each sex) and differences in sex ratios between seasons and generations at each sampling site were assessed using G-tests for goodness-of-fit and independence, respectively (Sokal and Rolf 1995). Variables were tested for normality using the Shapiro-Wilk W test. Non-normal data were transformed when necessary ($\log_{10}$, square root, or arcsine square root). Correlation matrices and associated scatter plots were used to assess linearity in relationships between proportions of females at each site and land use and environmental variables, proportions of females and snail population characteristics (parasitism and density), as well as correlations among land use and environmental variables. Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC), with a significance level of $\alpha = 0.05$.

In order to avoid errors associated with the use of linear regression for a binomial response (Sokal and Rolf 1995, Wilson and Hardy 2001), logistic regression (proc logistic in SAS, events/trials notation) was used to test hypothesized relationships between proportions of females and independent variables (as outlined in the study objectives), the validity of linear relationships indicated by correlation analysis, and relationships between categorical or non-normal independent variables and proportions of females. Stepwise logistic regression (model entry criterion: $P = 0.15$) was used for exploratory analysis of effects of combinations of uncorrelated land use and environmental variables on the measured proportion of females. For logistic regression models, Wald χ^2 statistics were used to assess significance of parameters, the concordance index (c) was used to assess predictive capability, Pearson residuals were used to assess model fit, and apparent overdispersion was assessed using Pearson's χ^2/degrees of freedom (SAS Institute, 1995, Wilson and Hardy 2001). If values for Pearson's χ^2/degrees of freedom exceeded two, and the model met criteria for overdispersion as outlined in SAS Institute (1995), overdispersion was corrected using the Williams method. This method was selected due to the different numbers of snails utilized to determine population sex ratios (SAS Institute 2010). The potential influence of outliers in the dataset was assessed using plots of Pearson χ^2 deletion differences and DfBetas (SAS Institute, 1995); none were influential and all were retained.

RESULTS

Tributary sites

A total of 14,887 snails from Shenandoah River tributaries were included in sex ratio calculations (Table 2). The proportion of females at each site during each sampling period ranged from 0.36–0.94, and overall mean proportions ranged from 0.46–0.87 (Table 2). Sex ratios at nine sites were consistently female-biased; proportions of females were significantly higher than 0.5 (G-test, $p \leq 0.020$) during all four sampling periods (Table 2). One site (Linville Creek) had significantly female-biased sex ratios during only the spring 2008 sampling period (G-test, $p = 0.0002$, Table 2). Four sites did not show any significant female bias during the four sampling periods, and two of these sites (Naked Creek and Cedar Creek DS) had significantly male-biased sex ratios during the spring 2008 sampling period (G-test, $p \leq 0.025$, Table 2). Along Cedar Creek, the sex ratio shifted from consistently female-biased at Cedar Creek US to balanced (or male-biased) at Cedar Creek DS, indicating a longitudinal shift within the same tributary (Table 2). The opposite pattern was observed between the US and DS sites on Hawksbill Creek (Table 2).

All but four sites had statistically similar proportions of females across the four sampling periods (G-test of independence, Table 2). Significantly different proportions of females during one or more sampling periods were observed at Stony Creek US, Muddy Creek, and Jennings Branch. However, these changes did not cause deviation from the overall trend of female-biased sex ratios at these sites during all sampling periods (Table 2). Hawksbill Creek US was the only site with fluctuating sex ratios. This site had a significantly male-biased population sex ratio during one sampling period (2007 generation, spring 2009) and a significantly female-biased population sex ratio during the next sampling period (2008 generation, fall 2009). There was also a large increase in the snail population density at this site during the last sampling period, which may reflect population instability. Overall, there was no pattern of different sex ratios between generations or seasons (2007 generation). Evaluation of sex ratios at two sampling sites for 16 months further indicated no

Table 2. Proportions of females in *Leptoxis carinata* populations in Shenandoah River tributaries during seasonal sampling of three generations of snails, including the total numbers of snails sampled (in parentheses), and the overall mean proportions of females ± one standard deviation (*SD*), and results of G-tests of independence.

#	Site Name	May 2008 (2006 gen.)	Oct. 2008 (2007 gen.)	May 2009 (2007 gen.)	Oct. 2009 (2008 gen.)	Mean ± *SD*	G-value
1	Cedar Cr.-US	0.65 (148)	0.63 (185)	0.75 (92)	0.65 (133)	0.67 ± 0.05	4.2
2	Cedar Cr.-DS	*0.40* (125)	0.52 (218)	0.56 (130)	0.52 (315)	0.50 ± 0.07	7.8
3	Stony Cr.-US	0.72 (167)	0.58 (173)	0.69 (223)	0.65 (720)	0.66 ± 0.06	8.5*
4	Stony Cr.-DS	0.71 (83)	0.74 (316)	0.72 (229)	0.65 (344)	0.70 ± 0.04	6.3
5	Passage Cr.	0.55 (134)	0.51 (163)	0.52 (123)	0.50 (325)	0.52 ± 0.02	1.0
6	Mill Cr.	0.68 (97)	0.61 (175)	0.62 (241)	0.61 (245)	0.63 ± 0.03	1.8
7	Smith Cr.	0.68 (170)	0.63 (209)	0.67 (513)	0.60 (533)	0.65 ± 0.03	6.4
8	Hawksbill.-US	0.56 (201)	0.52 (157)	*0.41* (190)	0.60 (875)	0.52 + 0.08	24**
9	Hawksbill-DS	0.67 (134)	0.61 (224)	0.60 (648)	0.59 (526)	0.62 ± 0.04	3.2
10	Long Meadow R.	0.89 (127)	0.85 (142)	0.82 (255)	0.79 (126)	0.84 ± 0.04	5.3
11	Linville Cr.	0.60 (135)	0.56 (139)	0.53 (94)	0.55 (110)	0.56 ± 0.03	1.3
12	Naked Cr.	*0.36* (83)	0.49 (143)	0.48 (103)	0.52 (148)	0.46 ± 0.07	5.7
13	Briery Br.	0.46 (124)	0.50 (280)	0.50 (545)	0.47 (460)	0.48 ± 0.02	1.3
14	Muddy Cr.	0.68 (148)	0.58 (543)	0.58 (411)	0.55 (693)	0.60 + 0.06	8.8*
15	Jennings Br.	0.79 (28)	0.92 (180)	0.83 (60)	0.94 (326)	0.87 ± 0.07	47**

Underlined proportions indicate significantly female-biased sex ratios (G-test, $p \leq 0.04$)
Italicized proportions indicate significantly male-biased sex ratios (G-test, $p \leq 0.025$)
G-values for G-test of independence: *$p < 0.05$, **$p < 0.0001$

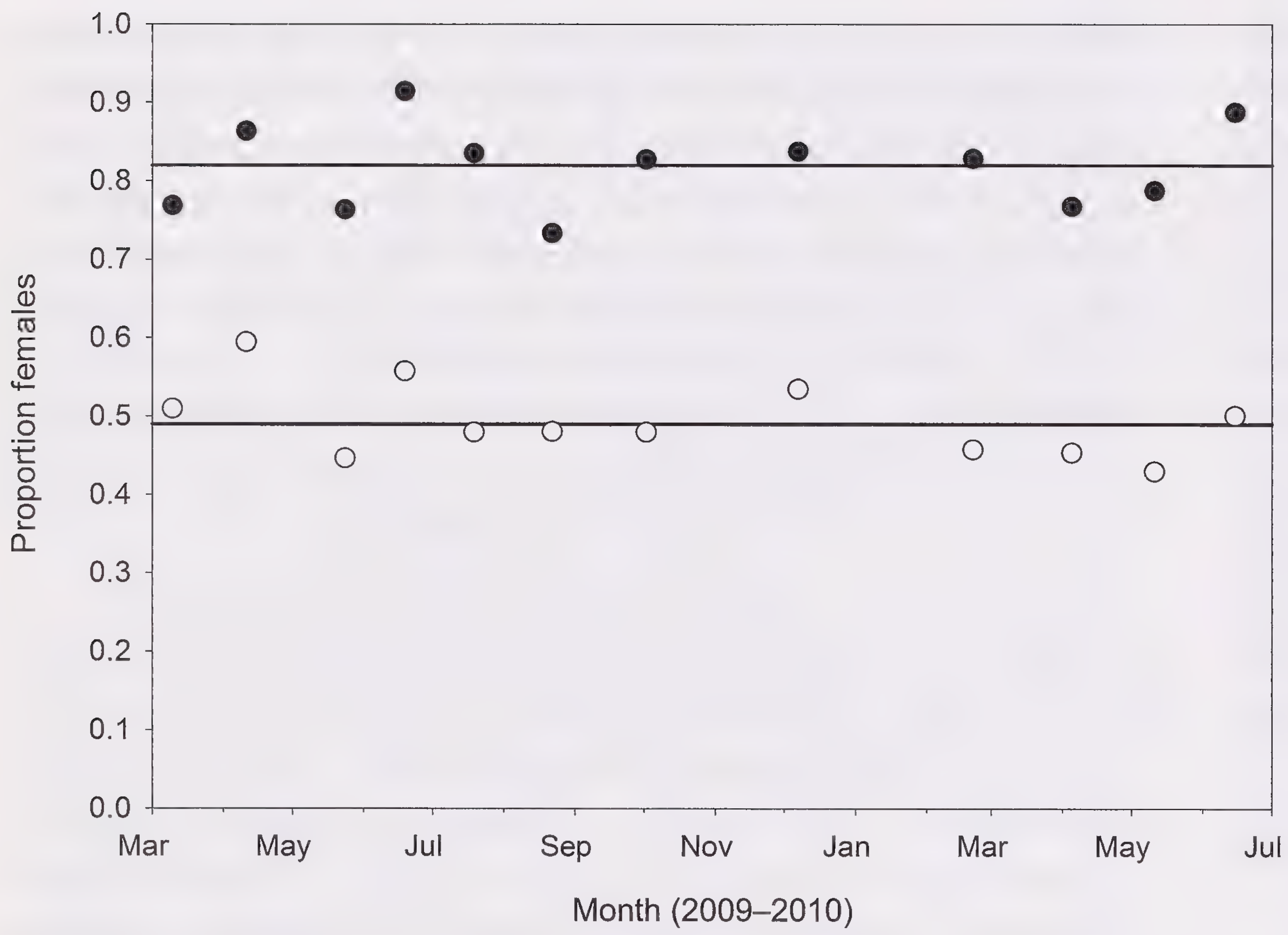

Figure 2. Variation in the proportions of females at two Shenandoah River tributary sites over a 16-month period, Briery Branch (open) and Long Meadow Run (filled). Lines represent the overall mean proportion of females at each site.

seasonal changes in proportions of females, as the variation in proportions of females between months was minimal (Fig. 2).

All calculated proportions of females at each site were included in analyses of relationships between sex ratios and land use, environmental variables, and other snail population characteristics. Contrary to the primary hypothesis, there were no linear relationships between proportions of females at tributary sites and mean concentrations of 17β-estradiol equivalents or the watershed density of AFOs ($p \geq 0.097$). The relationship between proportions of females at tributary sites and mean concentrations of 17β-estradiol equivalents was nonlinear (Fig. 3), but the shape of the curve (3rd order polynomial) did not indicate biological significance. There was a similar nonlinear relationship between proportions of females at tributary sites and watershed densities of AFOs. There were no apparent nonlinear relationships between the proportions of females at tributary sites and any other land use or environmental variables. At the landscape scale, the presence of WWTPs did not have a significant relationship with proportions of females in *Leptoxis carinata* populations at tributary sites ($P = 0.88$). However, the only major facility (discharge > 1 MGD) included in the study design was located on Hawksbill Creek, between the US and DS sites, and sex ratios did shift from primarily balanced at the US site to consistently female-biased at the DS site (Table 2).

The only variable with a significant linear relationship to the proportions of females at tributary sites was mean summer temperature ($r = -0.61$, $p < 0.0001$). This relationship remained significant when modeled with logistic regression (coefficient = -0.227 ± 0.036, Wald $\chi^2 = 39.66$, $p < 0.0001$, $c = 0.576$) (Fig. 4). Stepwise variable selection did not result in the inclusion of any other variables in the logistic regression model.

For *Leptoxis carinata* populations at tributary sites, densities of adults ranged from 75–2,332 snails/m^2, densities of juveniles ranged from 8–2,332 snails/m^2, and rates of parasitic infection were 2–77%. When compared to other snail population characteristics, there were no apparent nonlinear relationships or significant linear relationships between the proportions of females at tributary sites and adult densities ($P = 0.41$) or the proportions of trematode-infected snails ($P = 0.93$). There was no evidence that juvenile densities soon after hatching (2008 generation collected October 2008) were related to eventual proportions of mature adult females (2008 generation collected October 2009) in *L. carinata* populations ($P = 0.080$). There was also no evidence that proportions of adult females in *L. carinata* populations (2006 generation collected May 2008 and 2007 generation collected May 2009) were related to the densities of juveniles soon after hatching (2008 generation collected October 2008 and 2009 generation collected October 2009) ($P = 0.10$ and $P = 0.092$, respectively).

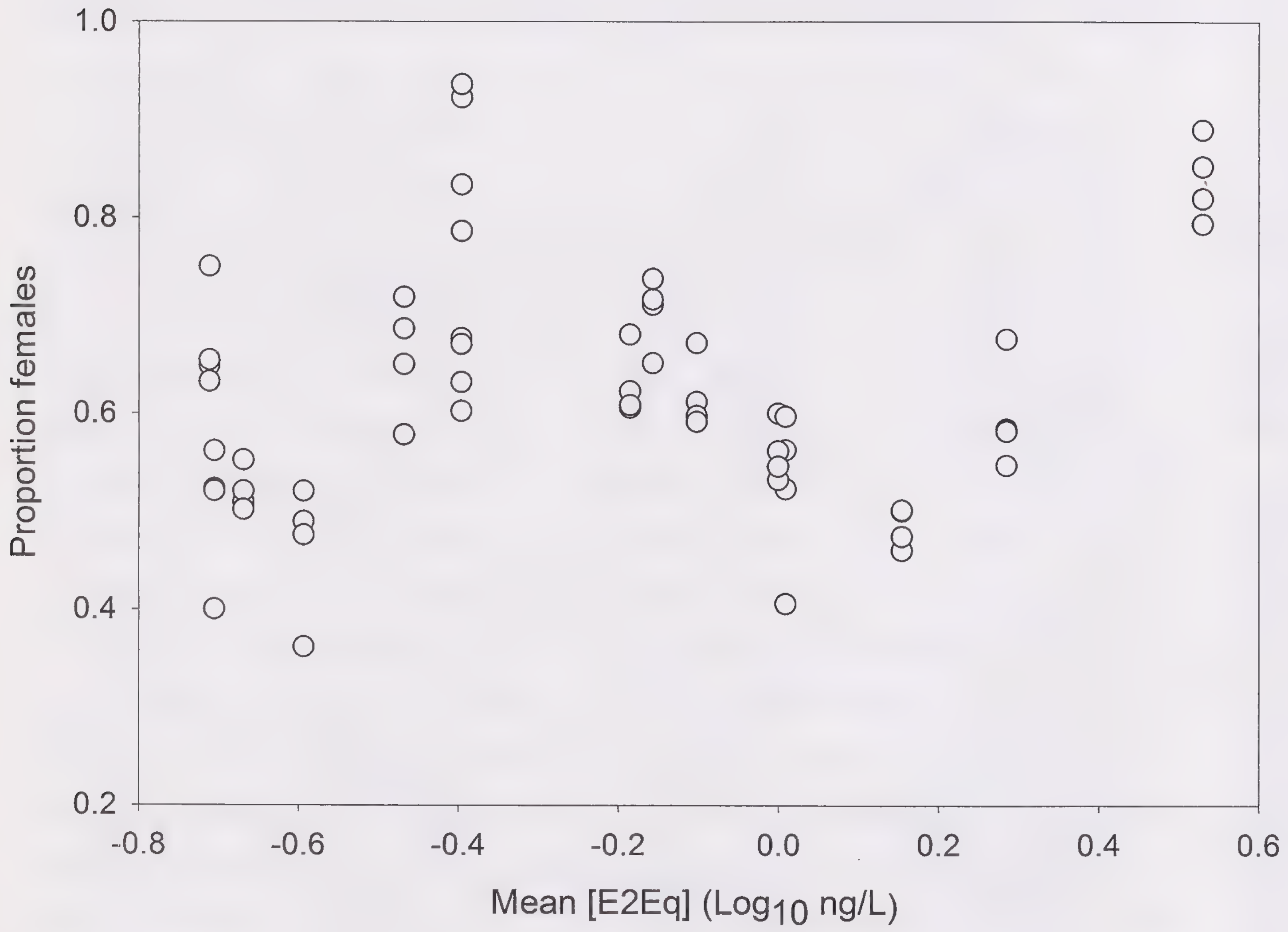

Figure 3. Apparent nonlinear relationship between the proportions of females at Shenandoah River tributary sampling sites and estrogenic activity as 17β-estradiol equivalents (E2Eq).

There were no significant relationships between proportions of female *Leptoxis carinata* at tributary sites and proportions of individuals in any of the shell

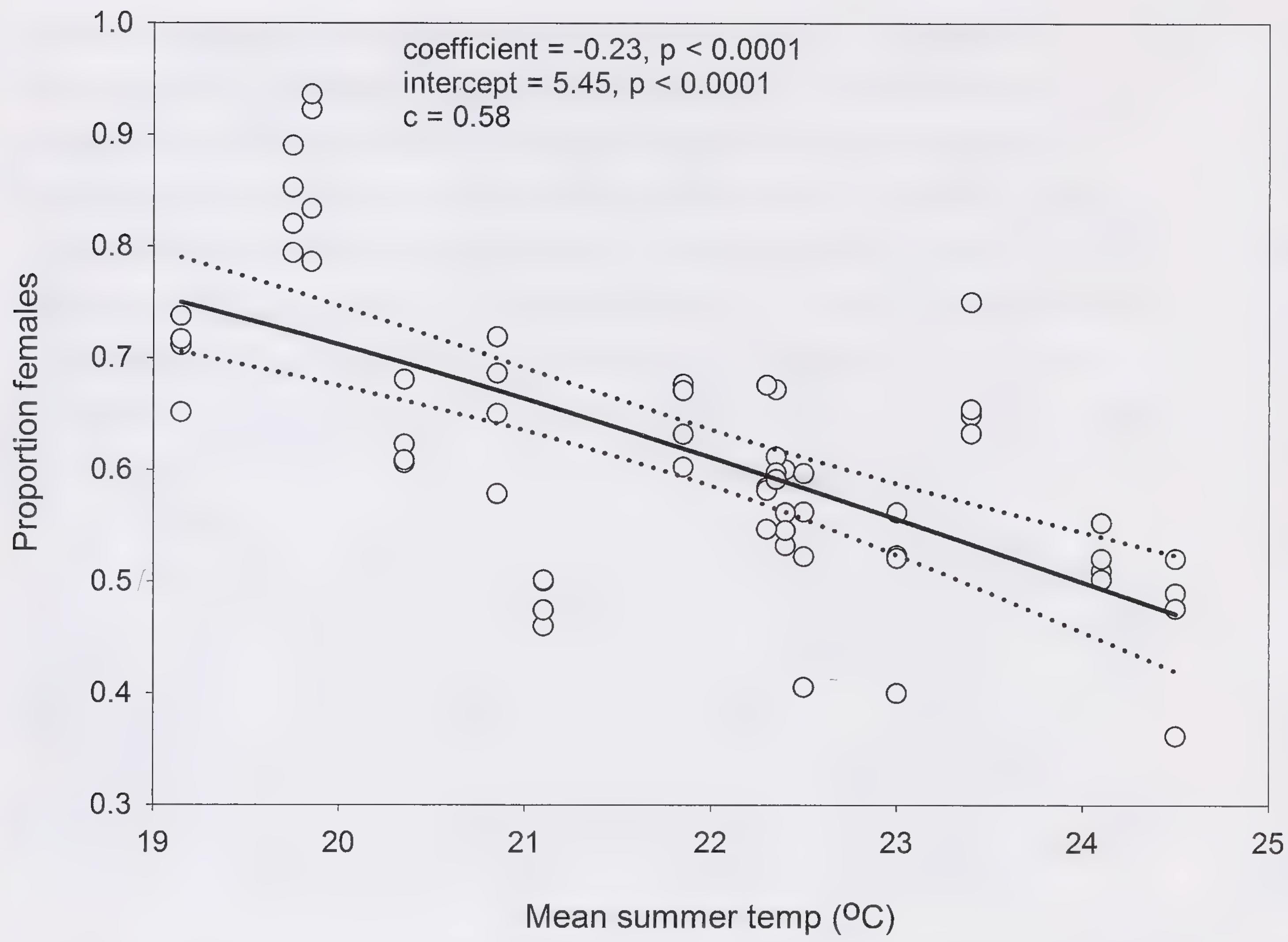

Figure 4. Relationship between proportions of females at Shenandoah River tributary sampling sites and mean summer temperature. The solid line represents predicted values from logistic regression and dotted lines represent upper and lower 95% confidence intervals. The coefficient, intercept, and concordance index (c) for the logistic model are listed.

length classes (1 mm intervals for 7–12 mm and > 12 mm) ($p \geq 0.26$). For the two tributaries evaluated over a 16-month period, proportions of females were not significantly different between 1-mm size classes from 7–12 mm (Briery Branch: $P = 0.11$ and Long Meadow Run: $P = 0.97$).

River sites

A total of 4,018 snails within the genus *Leptoxis* were included in calculations of sex ratios at river sites (Table 3). Proportions of females ranged from 0.37–0.82 during the two sampling periods (Table 3). Sex ratios at three sites were consistently female-biased; proportions of females at the James River site (I) and the two mid-river sites on the North Fork of the Shenandoah River (B and C) were significantly different from the expected proportion of 0.5 (G-test, $p \leq 0.0008$) during both sampling periods (Table 3). Proportions of females in populations of *Leptoxis* spp. were less than 0.5 at the Holston River, New River, and Rappahannock River sites, but sex ratios were significantly male-biased (G-test, $p = 0.0001$) at only the New River site during fall 2008 (Table 3). For all sites, the proportions of females were statistically similar between the two sampling periods (G-test of independence, G-values < 1, $p \geq 0.34$).

Measured proportions of females from both sampling periods were included in analyses of relationships between sex ratios and environmental variables and other snail population characteristics at river sites. Similar to tributary sites, the relationship between proportions of females and mean concentrations of 17β-estradiol equivalents at river sites was not significant ($P = 0.31$). The only environmental variable with a significant linear relationship to proportions of females at river sites was summer specific conductivity ($r = 0.76$, $p < 0.0001$). This relationship remained significant when modeled with logistic regression (coefficient = 0.0030 ± 0.0006, Wald $\chi^2 = 25.76$, $p < 0.0001$, $c = 0.631$) (Fig. 5). The predictive capability and fit of the model were not improved through stepwise addition of variables.

For *Leptoxis* spp. populations at river sites, densities of adults ranged from 60–1,992 snails/m^2, densities of juveniles ranged from 5–1,045 snails/m^2, and rates of parasitic infection

Table 3. Proportions of females in *Leptoxis* spp. populations in Virginia rivers during seasonal sampling of two generations of snails, including the total numbers of snails sampled (in parentheses), and the overall mean proportions of females ± one standard deviation (*SD*).

Code	River	*Leptoxis* spp.	May 2008 (2006 gen.)	Oct 2008 (2007 gen.)	Mean ± *SD*
A	NF Shenandoah	*L. carinata*	0.45 (42)	0.50 (160)	0.48 ± 0.03
B	NF Shenandoah	*L. carinata*	0.64 (144)	0.67 (444)	0.65 ± 0.02
C	NF Shenandoah	*L. carinata*	0.65 (167)	0.69 (177)	0.67 ± 0.03
D	NF Shenandoah	*L. carinata*	0.50 (131)	0.50 (142)	0.50 ± 0.00
E	North River	*L. carinata*	0.58 (117)	0.53 (226)	0.55 ± 0.04
F	SF Shenandoah	*L. carinata*	0.50 (262)	0.52 (242)	0.51 ± 0.02
G	Rappahannock	*L. carinata*	0.44 (94)	0.43 (183)	0.43 ± 0.00
H	Cowpasture R.	*L. carinata*	0.57 (138)	0.52 (187)	0.55 ± 0.04
I	James R.	*L. carinata*	0.82 (147)	0.81 (308)	0.82 ± 0.01
J	New R.	*L. dilatata*	0.42 (130)	*0.37* (238)	0.40 ± 0.03
K	Holston R.	*L. subglobulosa*	0.46 (153)	0.46 (186)	0.46 ± 0.00

Underlined proportions indicate significantly female-biased sex ratios (G-test, $p \leq 0.0008$)
Italicized proportion indicates significantly male-biased sex ratio (G-test, $p < 0.0001$)

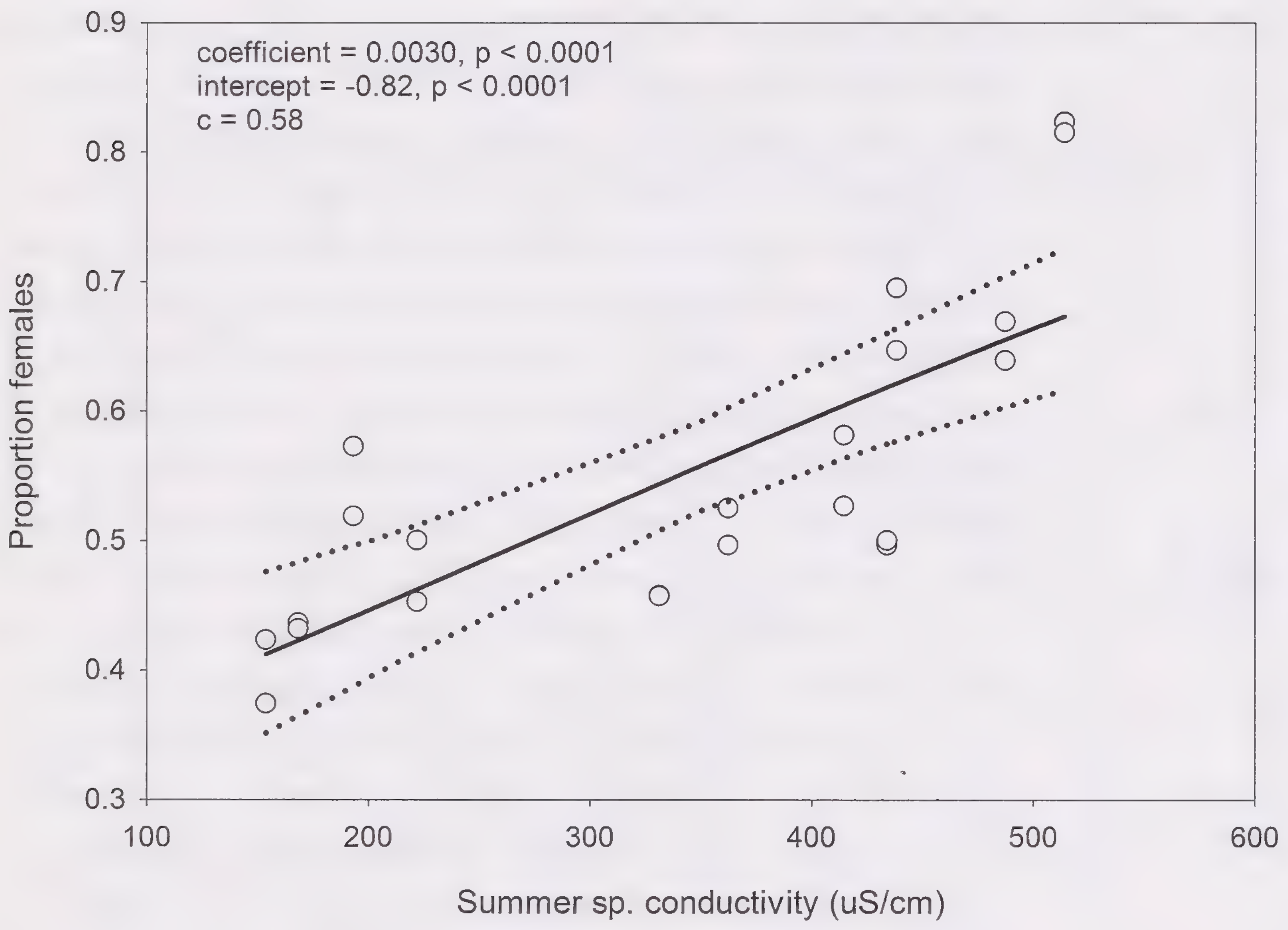

Figure 5. Relationship between proportions of females at river sampling sites and summer specific conductivity. The solid line represents predicted values from logistic regression and dotted lines represent upper and lower 95% confidence intervals. The coefficient, intercept, and concordance index (c) for the logistic model are listed.

were 0–79% .When compared to other snail population characteristics at the river sites, there were no significant linear relationships between the proportions of females and densities of adults ($P = 0.10$) or the proportions of snails infected with trematodes ($P = 0.50$). Proportions of females in *Leptoxis* spp. populations at river sites in May 2008 were not related to the density of juveniles soon after hatching (October 2008, $P = 0.83$).

DISCUSSION

The variation in proportions of females in *Leptoxis carinata* populations between sampling sites in Shenandoah River tributaries suggests that sex ratios were influenced by site-specific factors. Site-specific effects were further indicated by longitudinal changes in sex ratios between upstream and downstream site pairs in two Shenandoah River tributaries (Cedar Creek and Hawksbill Creek), and at sites in both the James River basin and North Fork of the Shenandoah River. With the exception of one site in the James River basin, snail population sex ratios at all sampling sites outside of the Shenandoah River basin were not female-biased. Previous studies of populations of *Leptoxis* spp. and other pleurocerid snails also found sex ratios ranging from balanced to female-biased (Aldridge 1982, Hendrix 1986, Miller-Way and Way 1989, reviewed by Dillon 2000), but sampling was generally not conducted over multiple seasons and snail generations at multiple sites. The low within-site variation in proportions of females in *Leptoxis* spp. populations between seasons and snail generations indicates consistency in the site-specific factors responsible for variation in population sex ratios. These site-specific factors did not appear to be related to food availability, resource competition, or infection by digenetic trematodes, as there were no significant relationships between proportions of females and benthic chlorophyll a concentrations, nutrient concentrations, landscape sources of nutrients, snail population densities, or trematode infection rates. However, there were significant relationships between proportions of females and abiotic environmental variables. These results suggest that environmental conditions could potentially affect either sexual differentiation or sex determination of *Leptoxis* spp., leading to spatial variation in population sex ratios.

Sexual differentiation

Exposure of individuals to contaminants during critical windows of development can result in disruption of normal sexual differentiation, phenotypic sex expression that differs from genotypic sex, and ultimately, biased population sex ratios. The primary hypothesis of this study was based on the idea that secondary sex ratios of *Leptoxis* spp. could be altered as a result of disruption of sexual differentiation by estrogens and estrogenic compounds. However, there were no significant relationships between proportions of females in *Leptoxis* spp. populations and measured estrogenic activity at tributary or river sites, or landscape sources of compounds contributing to estrogenic activity at tributary sites. One possible explanation is that estrogenic activity was only quantified during five sampling periods at tributary sites and two sampling periods at river sites. Variability in concentrations of estrogens and estrogenic compounds over the time period corresponding to hatching through gametogenesis in *Leptoxis* spp. (i.e. August–March; Ciparis 2011) could be more important than general trends in concentrations in terms of potential site-specific effects on sexual differentiation and population sex ratios. Exposures of fish to natural and synthetic estrogens have shown that the timing and duration of exposure is related to the degree of complete feminization within the exposed populations (Hahlbeck *et al.* 2004, Fenske *et al.* 2005, Lange *et al.* 2009).

Alternatively, sexual differentiation in *Leptoxis carinata* may not be susceptible to feminizing effects of estrogens. In a study of oysters, the xenoestrogen nonylphenol was an apparent cause of female-biased sex ratios in adult populations after exposure of undifferentiated larvae (Nice *et al.* 2003), but the actual role of steroidal estrogens in sexual differentiation of mollusks is unknown. Injections of oysters with 17β-estradiol during early stages of seasonal maturation induced sex reversal from males to females and resulted in female-biased sex ratios (Mori *et al.* 1969). In contrast, injections of undifferentiated juvenile scallops with 17β-estradiol stimulated morphological differentiation of gametes, but ultimately resulted in significantly more males than females relative to unexposed scallops (Wang and Croll 2004). Aqueous exposures of undifferentiated mollusks to environmentally realistic estrogen concentrations, with subsequent evaluation of effects on sexual differentiation, have yet to be conducted.

Sexual differentiation of *Leptoxis carinata* could potentially be disrupted by non-estrogenic pathways and several non-estrogenic contaminants are present in the study area. Relatively high concentrations of arsenic (1.2–10 μg/g dry wt.) have been measured in tissue from *L. carinata* and Asian clams from Shenandoah River tributaries, and several potential minor sources of arsenic are present in the Shenandoah River watershed (Ciparis *et al.* in press). Agricultural pesticides including: atrazine, simazine, and metolachlor, have been detected in groundwater and streams within the Shenandoah River watershed (Ator *et al.* 1998). In passive samplers deployed in the Shenandoah River, concentrations of these agricultural pesticides were highest at a North Fork Shenandoah River site corresponding to the location of site B in the current study (Alvarez *et al.* 2008). Outside of the Shenandoah River watershed, a tributary of the James River (Jackson River) upstream of site I receives effluent from a large pulp and paper mill, and the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in bleach kraft mill effluent is well documented (USEPA 2007). Arsenic, agricultural pesticides, and 2,3,7,8-TCDD may interfere with retinoid signaling systems (Nilsson and Hakansson 2002, Berube *et al.* 2005, Boily *et al.* 2005, Davey *et al.* 2008). Retinoic acid signaling via the retinoid X receptor (RXR) is a probable mechanism for development of male characteristics in female marine gastropods (imposex) after exposure to tributyltin, as inappropriate timing of RXR activation by tributyltin during reproductive development appears to stimulate development of imposex (Nishikawa *et al.* 2004, Castro *et al.* 2007, Sternberg *et al.* 2008). Therefore, it seems plausible that interference with retinoic acid signaling or direct suppression of RXR could suppress male development and stimulate development of female characteristics, although involvement of this signaling system in sexual differentiation of immature gastropods or other mollusks has yet to be studied.

The relationships between proportions of females in *Leptoxis* spp. populations at tributary and river sites and abiotic environmental variables may indirectly relate to the relative exposure of *Leptoxis* spp. to contaminants. Mean summer temperature was negatively related to proportions of females in *L. carinata* populations at tributary sites. Many streams in the Shenandoah River watershed are underlain by carbonate bedrock and spatial variation in groundwater contribution to streamflow is common in carbonate systems (Lindsey *et al.* 2003, Yager *et al.* 2008). Carbonate geology also creates the potential for rapid transport of contaminants from landscape sources to streams via groundwater (Ator *et al.* 1998). Thus, in addition to lower summer temperatures, *L. carinata* populations at sampling sites closer to groundwater discharges could also experience more consistent exposure to contaminants in groundwater or exposure to higher concentrations of these contaminants relative to populations at sampling sites further from groundwater discharges.

Summer specific conductivity was positively related to the proportions of females in *Leptoxis* spp. at river sites. Elevated conductivity in streams and rivers can occur as a result of runoff from agricultural and urban landscapes (Price and Leigh 2006, Walters *et al.* 2009) and discharge of effluent from municipal and industrial WWTPs (Hall *et al.* 2009, Gagne *et al.* 2011). In the North Fork of the Shenandoah River, major WWTP discharges and several tributaries draining areas of intensive agricultural production enter the river between sites A and C, which corresponds to a shift in *L. carinata* population sex ratios from balanced to female-biased. As previously described, the tributary receiving bleached kraft mill effluent from a pulp and paper mill enters the James River between sites H and I, which also corresponds to a shift in population sex ratios of *L. carinata*. In addition to ions, runoff and effluent discharges can introduce a number of contaminants to streams and rivers, which creates a potential mechanism for an indirect relationship between specific conductivity and proportions of females in *Leptoxis* spp. populations at river sites.

Sex determination

The significant negative relationship between mean summer temperature and proportions of females in *Leptoxis carinata* populations at tributary sampling sites suggests the possibility of an environmental influence on sex determination in *L. carinata*. Temperature dependent sex determination is a type of environmental sex determination that is well described in several species of fish and reptiles (Bull 1983, Conover 2004), but is not well studied in invertebrates. In populations of Atlantic silverside, *Menidia menidia*, offspring produced early in the breeding season when water temperatures are low primarily develop as females and grow larger than offspring produced later in the breeding season, which develop primarily as males (Conover 2004). Earlier development of females relative to males could be advantageous for semelparous *L. carinata*, given the prolonged egg-laying period (April–July) and short period for growth prior to reproduction at 20–24 months of age (Aldridge 1982). However, multiple collections of *L. carinata* from a tributary with consistently balanced population sex ratios (Briery Branch) provided no indication of greater proportions of females in larger size classes. Similar results were obtained from a single collection of another pleurocerid snail, *Elimia proxima* (Say, 1825), from a population in a North Carolina river with a balanced sex ratio (Dillon 2000). Thus, it appears as if early development of females does not occur in populations of *L.carinata and E. proxima*. Water temperature is probably not directly related to sex determination in *L. carinata,* although thorough study of the mechanism of sex determination in pleurocerid snails would be required for a definitive conclusion.

Significance of female-biased sex ratios?

The significance of female-biased sex ratios within populations of pleurocerid snails is unknown. There were no significant relationships between proportions of females in *Leptoxis* spp. populations and densities of juvenile snails. This suggests that greater proportions of females within populations do not result in greater production of surviving offspring. *Leptoxis carinata* females do produce fewer eggs in response to higher egg densities (Aldridge 1982), and given the very low survival rate of eggs, there could be a cost to individual fitness. Similarly, individual fitness could be reduced in populations with strongly female-biased population sex ratios due to increased competition for mates. It is unclear whether populations with strongly biased sex ratios are unhealthy. Overproduction of one sex is adaptive in some species of birds and mammals, but characteristics of *L. carinata* do not meet the criteria for expected departure from equal gender allocation which include: low fecundity, high parental care, and intense sexual selection (Frank 1990). It is possible that overproduction of females within *Leptoxis* spp. populations at some sampling sites is an adaptive response to stress, but a mechanism for evolution and maintenance of this pattern is unknown. It is also possible that female-biased sex ratios at some sites are the result of increased prevalence or population susceptibility to parasites not evaluated in this study. Microsporidians can cause female-biased sex ratios through male-killing or inhibition of male development, and can be vertically transmitted within populations (Terry *et al.* 2004). Microsporidians have been identified in several species of pulmonate snails (McClymont *et al.* 2005), but their presence in pleurocerid snail populations has yet to be investigated and may warrant further study.

CONCLUSIONS

Results of this study provide some support for the general hypothesis that sexual differentiation in pleurocerid snails can be affected by environmental conditions. However, there was no evidence of an effect specific to compounds that can bind to the estrogen receptor. Given the current interest in assessing effects of endocrine-disrupting compounds on aquatic organisms, further research on mechanisms of sex determination and sexual differentiation in mollusks appears to be of critical importance. For the notoriously understudied species of gonochoristic freshwater gastropods, knowledge of the significance of sex ratio variation could potentially increase their utility as bioindicators and benefit their conservation, particularly if female-biased sex ratios are an indication of contaminant exposure or stressed populations.

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Shell characteristics of land snail *Eobania vermiculata* (Müller, 1774) (Helicidae) from Croatia

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Abstract. The land snail genus *Eobania* P. Hesse, 1913 in Croatia consists of four taxa: *Eobania vermiculata vermiculata* (O.F. Müller, 1774), *E. v. pelagosana* (Westerlund, 1894), *E. v. figarole* (Rensch, 1928) and *E. v. kamenensis* Berberović, 1963. Only *E. v. vermiculata* and *E. v. pelagosana* have been included in this study. The mean shell diameters (D) of the 13 samples ranged from 19.99 mm (sample from the island of Sušac) to 31.78 mm (Šibenik, seaside) and the mean shell height (H) from 17.31 mm (Sušac) to 25.66 mm (Island of Šolta). Shell morphology confirms that all island populations are smaller with examples of nanism on outside islands of Palagruža and Sušac. However, molecular analysis (16S rDNA and *COI* sequence data) showed no significant differences between the South Adriatic population from the mainland and islands, thus providing no molecular evidence for classification of a single *E. vermiculata* species into separate subspecies within the investigated area. The observed differences might possibly be attributed to ecological influences. The population with the largest average shell size was approximately 1.5x larger than the population with the smallest average size.

Key words: chocolate banded snail, morphometrics, nanism, island

Eobania vermiculata (Müller, 1774) or chocolate banded snail is a widely distributed land snail in Mediterranean countries. The nearest countries to Croatia in which *Eobania vermiculata* is registered are Italy, Bulgaria (Dedov 1998), Montenegro and Albania (Dhora 2009, Féher and Erőss 2009, Bank 2011), Greece (Welter-Schultes and Williams 1999) and Turkey (Örstan *et al.* 2005). However, non-indigenous populations of this species are already established in the U.S.A. and elsewhere around the world, where this species is considered to represent a potentially serious threat as a pest and invasive species (Cowie *et al.* 2009).

Our review of the available literature established that *Eobania vermiculata* has been recorded in Croatia only in general terms in Dalmatia (Jaeckel *et al.* 1957), on the island of Dugi otok (Štamol 2004, Štamol and Kletečki 2005, Štamol 2010) and as subspecies *Eobania vermiculata pelagosana* (Stossich, 1877) from the islands of Palagruža and Sušac (Berberović 1963, 1964) and *E. vermiculata figarolae* (Rensch, 1928) from the island of Figarola nearest to Rovinj. Molecular phylogeny and biogeography of the subspecies of *E. vermiculata* from Croatia are unknown.

Previous studies on terrestrial gastropods *Helix aspersa* (O. F. Müller, 1774) and *Eobania vermiculata* (Sacchi 1957; Berberović 1963, 1964) in Croatia were based only on morphometric characteristics of the shell. The results showed that all shells from the Adriatic islands are smaller than the average values for specimens from the mainland. La Graeca and Sacchi (1957) noted that the gastropod shell size on the small islands in the Mediterranean is different from the continental populations and the values are much smaller. This "nanism" is especially expressed in populations of the islands of Palagruža and Sušac (open sea islands close to the Italian shore), so Berberović (1964) described a new subspecies, *Helix aspersa pelagosana* in the genus *Helix*, and Stossich (1877) *Eobania vermiculata pelagosana* in the genus *Eobania*. The main reason for such phenomena is the origin of Adriatic islands which formed after the last glacial period (app. 10,000 years ago), after the rising of the sea level. Since then, the sea level has risenby approximately 100 meters (Šegota 1968, Šegota and Filipčić 1991). Because of the geographical isolation, the fauna of those islands is rich with numerous new species and subspecies awaiting discovery. In addition the climate features of that area represent another limiting factor (Trošić *et al.* 2003) in reaching a certain body size designated for the species. Past research of the flora on the island of Palagruža recorded an unusual finding of dwarfism in wild olives, *Olea europea* L., and tree spurge, *Euphorbia dendroides* L., described by Trinajstić (1973) as the *Oleo-Euphorbietum dendroides* community. Other authors (Palkovacs 2003, Filin and Ziv 2004) explained adaptive shifts in body size on islands, but on terrestrial vertebrate groups and plants. They stated that several factors influence increased as well as decreased body size, such as competition, predation, resource limitation and life history. The same model could be used on invertebrates as well.

The basic survey on *Eobania vermiculata* shell morphometry was made by Berberović (1963) on mainland and island populations from Central Dalmatia (Croatia). He identified three separate groups: 1. Group Palagruža and Sušac; 2. Group "Biševo" and 3. Group "Lastovo". Shell characteristics

Table 1. Morphometric shell characteristics of *Eobania vermiculata* according Berberović (1963): H, mean value of total shell height; D, mean value of shell width; ha, mean value of aperture height; H/D, ratio between total shell height and shell width.

1. Outer islands	H (cm)	D (cm)	ha (cm)	H/D (%)
Palagruža	1.620	2.239	1.156	72.50
Sušac	1.684	2.332	1.198	72.46
Lastovo	1.938	2.673	1.389	72.64
Biševo	1.713	2.324	1.189	73.70
Vis	1.834	2.567	1.287	71.44
2. Continental line (seaside)				
north to the river Cetina	2.001	2.931	1.481	68.57
between rivers Cetina and Neretva	2.021	2.899	1.500	69.96
south to the river Neretva	1.972	2.937	1.528	67.55
3. Inner islands				
Šolta	1.984	2.962	1.537	67.04
Brač	1.917	2.788	1.442	68.12
Hvar	1.932	2.799	1.439	69.15
Korčula	1.901	2.759	1.412	68.90

of Group Palagruža and Sušac showed the nanism caused by the geological origin of the respective islands, vegetation and abundance. The "Biševo" Group is more similar to that of the Island of Gali (Gulf of Salerno). He also divided all examined populations into morphological groups (Table 1) in relation to the populations from Palagruža and Sušac to "continental type" according to H/D ratio (ratio between height and width in percentages; lower H/D ratio than Palagruža and Sušac; central Adriatic islands except the island of Vis and the Lastovo archipelago). The Lastovo archipelago and the island of Vis belong to the "Lastovo type" (H/D ratio same or higher than Palagruža and Sušac), whereas "Biševo type" consists of populations from the island of Biševo, Korčula and Hvar (H/D ratio much lower than Palagruža and Sušac). The contact zone between the types is the island of Vis with the neighboring islands. Mainland populations are divided into populations north of the Cetina river, between the Cetina river and the Neretva river, and south of the Neretva river. In this paper we examined the same shell parameters as Berberović (1963), and we present the morphometric characteristics of 13 different populations of *E. vermiculata*. The aim was to determine if any changes in shell size took place in the period between Berberović (1963) and today.

MATERIAL AND METHODS

The sampling design used followed the pattern of Berberović (1963) (samples for the Central Adriatic) as well as from North and South Adriatic, fifteen sites in total (Figs. 1 and 2).

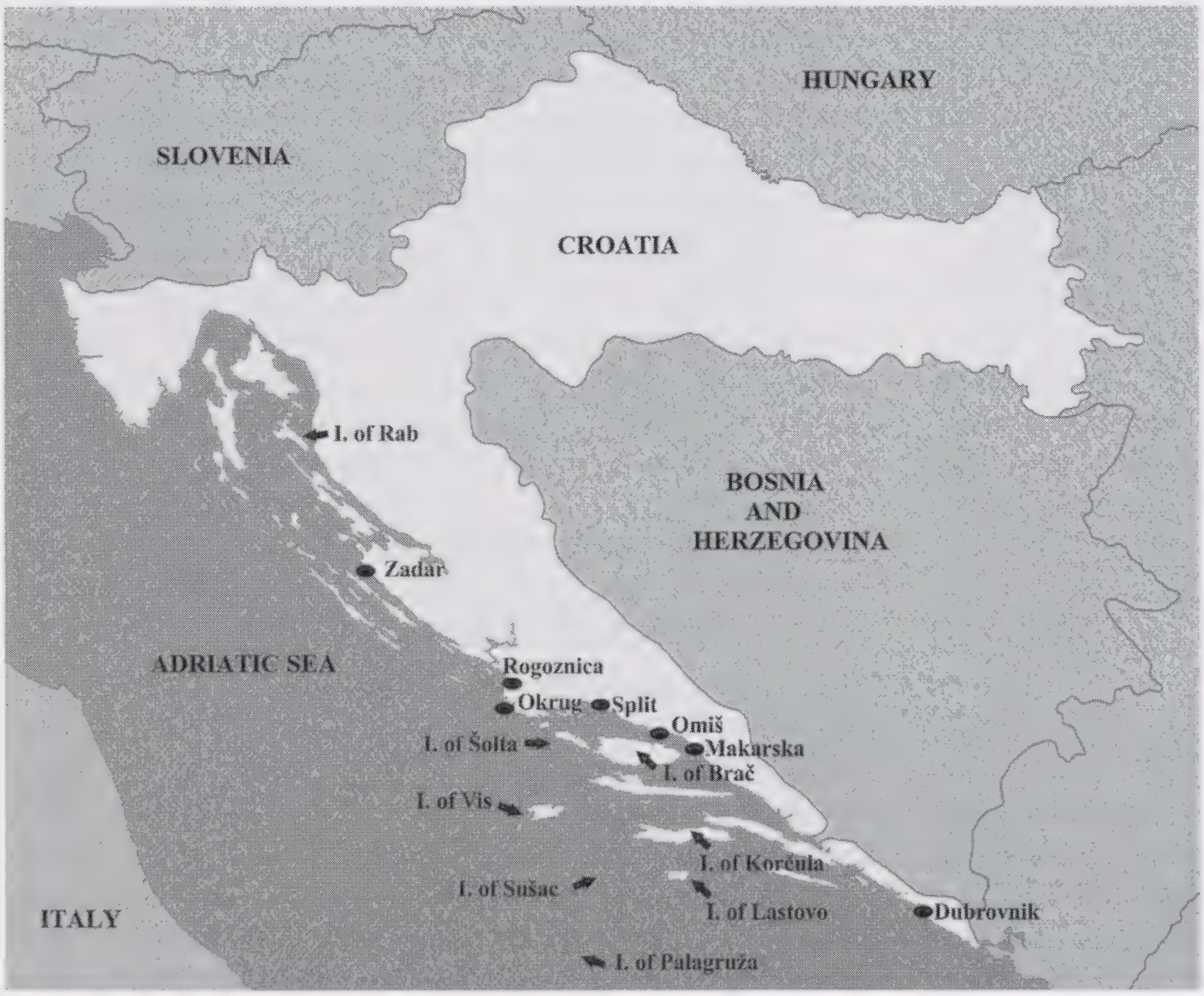

Figure 1. Map with sampling localities.

For shell measurements we collected 200 specimens per site, and a total of 2600 specimens with a reflected lip, because this indicates cessation of growth and maturity of the snail. The measurements were made in the field using vernier callipers. We measured shell height (H), shell width (D), and aperture height (ha). After that we compared the results as relative shell height (H/D) (ratio between height and width in percentages), and ratio between aperture height (ha) and shell height (H) in percentages. Fifty-nine adult individuals were sampled alive and transported in boxes to the laboratory, where we prepared a foot tissue clip from each individual for molecular analysis. All other specimens were returned to their natural habitats immediately after measurements.

The intrapopulation variation was calculated with the coefficient of variation (CV) for all the morphometric characteristics of the examined populations.

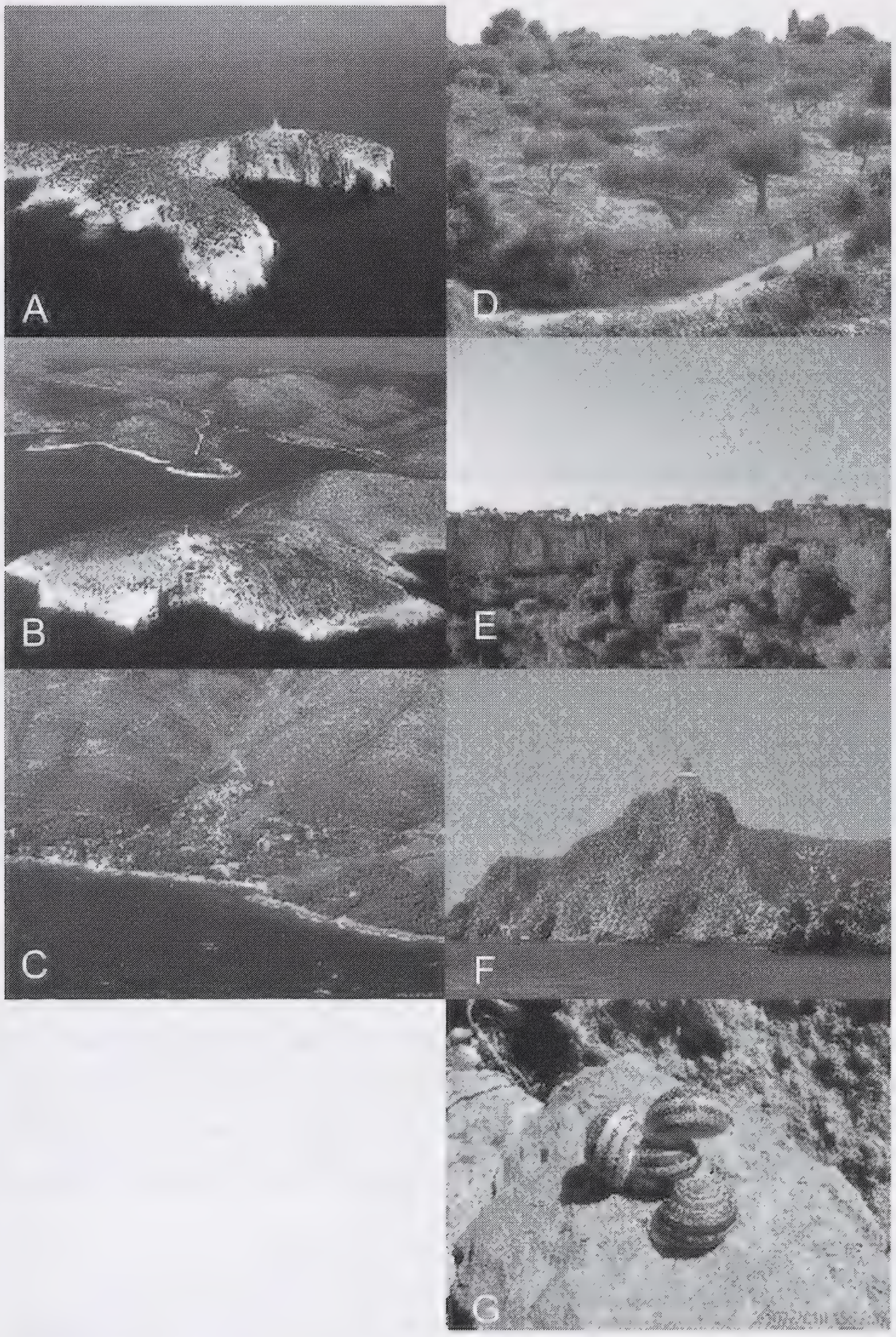

Figure 2. Collage of habitat photos of several sampling localities.

Table 2. Haplotypes and GenBank accession numbers for *Eobania vermiculata* 16S rRNA and *COI* sequences generated in this study and corresponding data from previously published studies.

	16S rRNA			***COI***		
Country	Haplotype	Accession number	*N*	Haplotype	Accession number	*N*
Croatia	H1	JF277380	31	H1	JF277391	11
	H2	JF277381	13	H2	JF277392	3
	H3	JF277382	4	H3	JF277393	1
	H4	JF277383	3	H4	JF277394	12
	H5	JF277384	2	H5	JF277395	2
	H6	JF277385	1	H6	JF277396	13
	H7	JF277386	1	H7	JF802030	7
	H8	JF277387	1	H8	JF802031	2
	H9	JF277388	1	H9	JF802032	2
	H10	JF277389	1	H10	JF802033	4
	H11	JF277390	1			
Total			59			57
Sienna		AY741409				
France		AY546357			AY546277	

Geographical variation of the collected specimens according to three variables of the shell (shell height, shell width and aperture height) was subjected separately to Principal Component Analysis (PCA). The UPGMA analysis was used to calculate similarities between the populations on 13 localities. All statistical calculations were made by Statistica 8.0 and XlstatPro softwares.

DNA-isolation, PCR-amplification and sequencing of DNA

The foot muscle of fifty nine adult individuals of *Eobania vermiculata* was dissected and DNA extracted according to Sokolov (2000). Amounts of 0.2–1 ng of total DNA (quantified on a 1% agarose gel) were used in polymerase chain reaction (PCR). Specific PCRs for partial 16S ribosomal DNA (rDNA) and mitochondrial cytochrome oxidase c subunit I (*COI*) gene were performed with the primers described in Steinke *et al.* (2004). DNA was purified using a QIAGEN Plasmid Mini Kit, and gel electrophoresis products by QIAEX II Gel Extraction Kit following manufacturer protocols. Samples were subjected to cycle sequencing using an ABI PRISM® 3100-Avant Genetic Analyzer (MACROGENE, Korea).

Data analysis

Forward and reverse sequencing products were aligned to generate a complete sequence and edited manually using BioEdit (Hall 1999). DNA sequences were aligned using ClustalW (Higgins *et al.* 1996), and refined manually. The obtained sequences were deposited in GenBank (accession numbers JF277380–JF277396 and JF802030–JF802030) (Table 2). The number of polymorphic sites, parsimony informative sites, haplotype and nucleotide diversity within (π) population as well as neutrality tests (Tajima's D and Fu's FS values) were determined using DnaSPv5 (Librado and Rozas 2010).

Phylogenetic relationships among investigated haplotypes were analyzed using Bayesian analysis implemented in MrBayes 3.1.1 (Huelsenbeck and Ronquist 2001). The model of nucleotide evolution that best fitted the data was determined by the Akaike Information Criterion (AIC) (Akaike 1974), as implemented in jModelTest 0.1.1 (Posada 2003, 2006). The 16S rRNA and *COI* trees were rooted using *Helix aspersa* 16S rRNA (GenBank GQ402387) and *H. aspersa COI* sequence (GenBank JN701927), respectively, as an outgroup. Haplotype networks were also built from the same data using TCS (Clement *et al.* 2000).

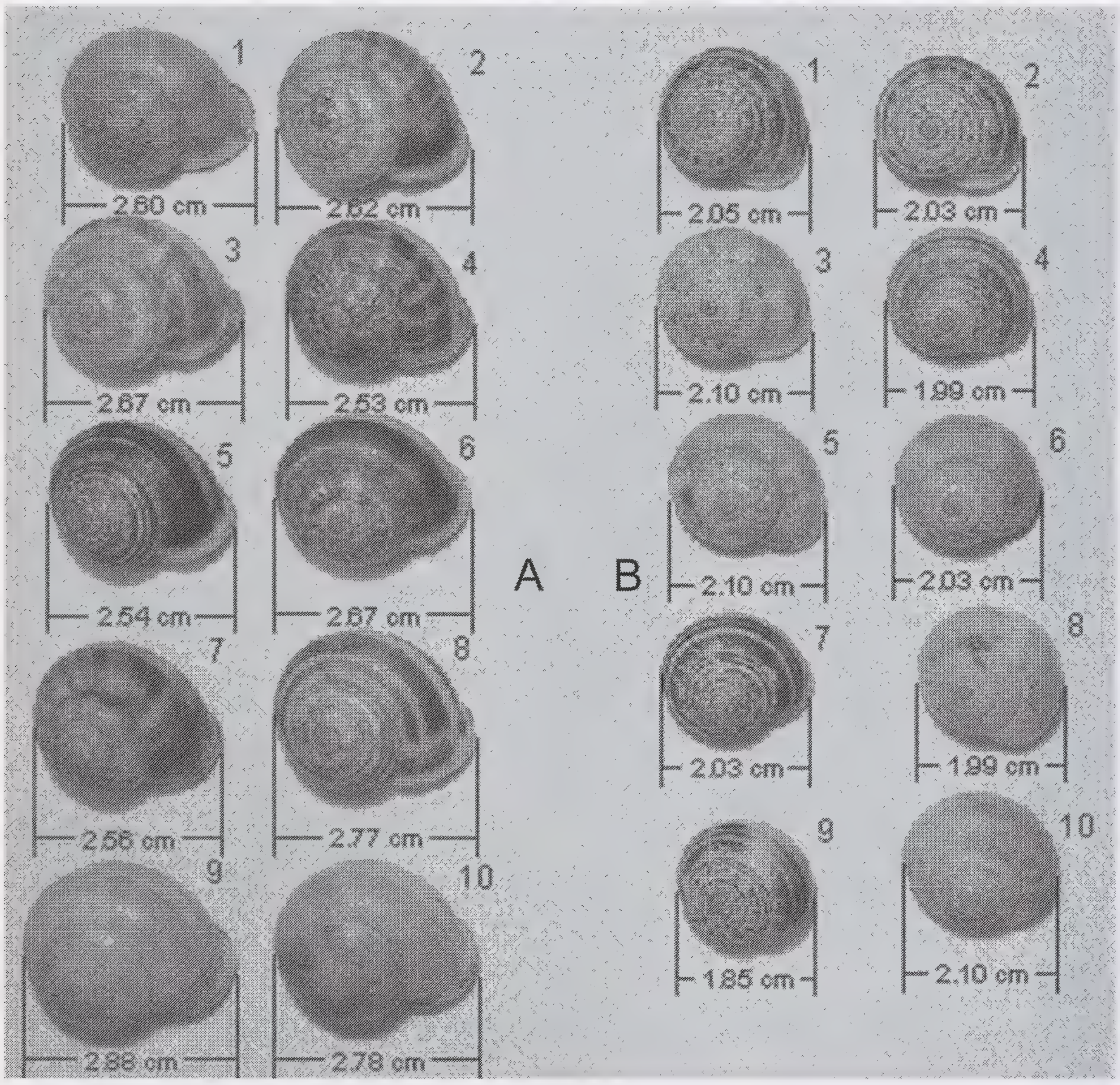

Figure 3. Empty shells with dimensions in cm. **A**, continental group and inner islands: 1–2, Split; 3–4, Vis; 5–6, Lastovo; 7–8, Dubrovnik; 9–10, Brač; **B**, Sušac and Palagruža: 1–6, Sušac; 7–10, Palagruža.

To infer the evolutionary relationship of the 16S RNA haplotypes obtained in the present study, we made comparison with sequences available in GenBank (AY546357) from Marseille, France (Steinke *et al.* 2004). To infer the evolutionary relationship of *COI* haplotypes obtained in the present study, we made a comparison with a single corresponding Genbank sequence (AY546277) from Marseille, France (Steinke *et al.* 2004).

The evolutionary history was inferred using the UPGMA method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide concatenated sequences. All positions containing gaps and missing data were eliminated. There were a total of 757 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura 2011).

RESULTS

Morphometric parameters

The mean shell diameters (D) of the measured samples ranged from 19.99 mm (sample from the island of Sušac) to 31.78 mm (Šibenik, shore) and mean shell height (H) from 17.31 mm (Sušac) to 25.66 mm (island of Šolta). The mean size of the population with the largest average shell size was approximately 1.5 × larger than the mean size of the population with the smallest average shell size (Fig. 3). Coefficient of

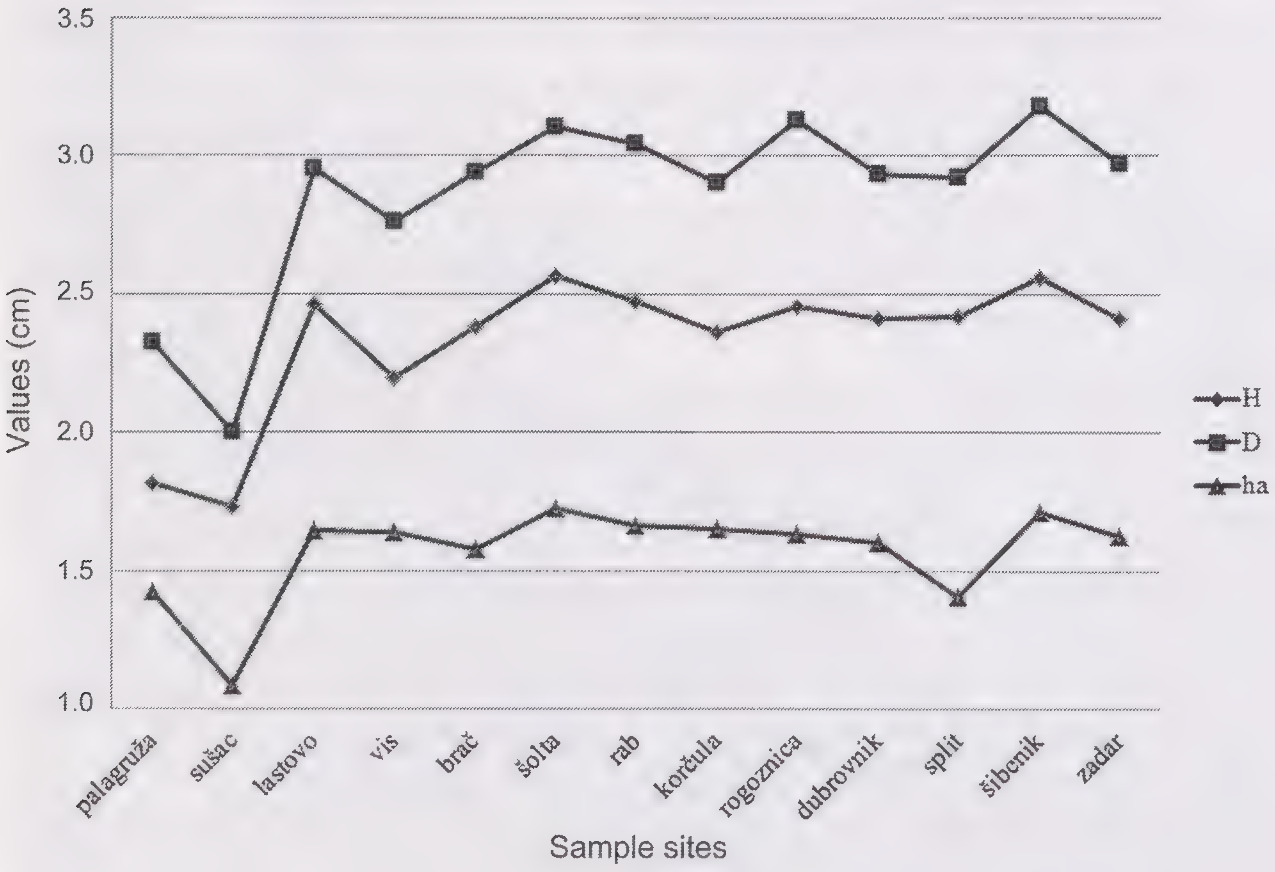

Figure 4. Diagram with three morphometric variables (**H**, shell height; **D**, shell width; **ha**, aperture height).

Table 3. The morphometric characteristics of *Eobania vermiculata* (H, shell height; D, shell width; ha, aperture height; *SD*, standard deviation, and CV, coefficient of variation).

sampling sites	H (cm)	D (cm)	ha (cm)
Palagruža	1.815	2.324	1.425
Sušac	1.731	1.999	1.087
Lastovo	2.461	2.956	1.644
Vis	2.199	2.76	1.636
Brač	2.382	2.941	1.575
Šolta	2.566	3.106	1.722
Rab	2.475	3.047	1.661
Korčula	2.365	2.903	1.651
Rogoznica	2.456	3.133	1.633
Dubrovnik	2.410	2.932	1.602
Split	2.42	2.92	1.404
Šibenik	2.558	3.178	1.714
Zadar	2.411	2.973	1.628
mean	2.327	2.859	1.568
median	2.411	2.941	1.633
min.	1.731	1.999	1.087
max.	2.566	3.178	1.722
SD	0.263	0.335	0.173
CV (%)	11.285	11.726	11.029

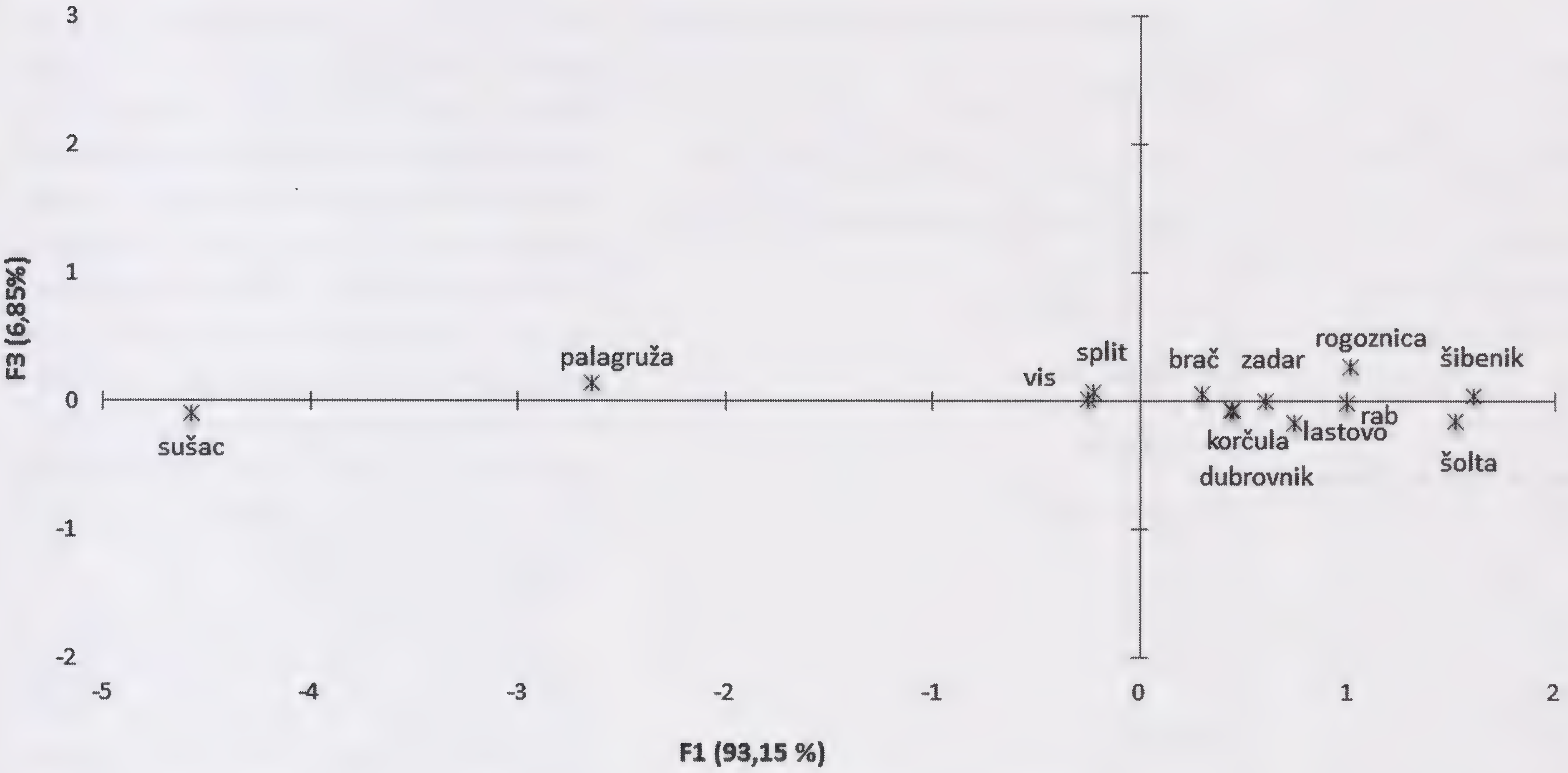

Figure 5. Principle Components Analysis (PCA) diagram (F1 represents H value-shell height, F3 represents ha value-aperture height)

variation (CV%) of the values of H, D and ha ranged from 11.02–11.72% and shows the low variance for the size variation within populations. The H/D ratio ranged from 0.78 (sample from the island of Palagruža) to 0.86 (sample from the island of Sušac) (Fig. 4). The low variance between the size variation within populations and variation in shell shape were not expected (Table 3).

The PCA analysis based on morphometric data of the shell characteristics of *Eobania vermiculata* confirmed that the populations from Islands of Palagruža and Sušac are clearly separate from the other populations (Fig. 5).

The variables which mainly contributed to the first discriminant function (squared canonical correlation 0.98%) were shell height (H) and, for the second discriminant function (squared canonical correlation 0.94%), shell width (D). The populations from the island of Palagruža and Sušac constituted a homogenous group (76.71%), populations from the island of Šolta and Šibenik (coast) constituted a more or less homogenous group at the level of 13.57% whereas those from other sites were heterogenous (0–3%).

The first factor (H, 93.15) and the second one (ha, 6.85%) separated populations into seven groups according to their geographic proximity. The first group is characterized by two populations: Palagruža and Sušac; the second group by the population from the island of Vis; the third by Split (shore); the forth by the island of Šolta and Šibenik (seaside); the fifth by the islands of Rab and Rogoznica (shore); the sixth by the island of Korčula; the seventh is divided into three subgroups: Zadar (shore), the island of Lastovo and Brač, and Dubrovnik (shore). UPGMA cluster analysis based on Euclidian distance (Fig. 6) was consistent with PCA results.

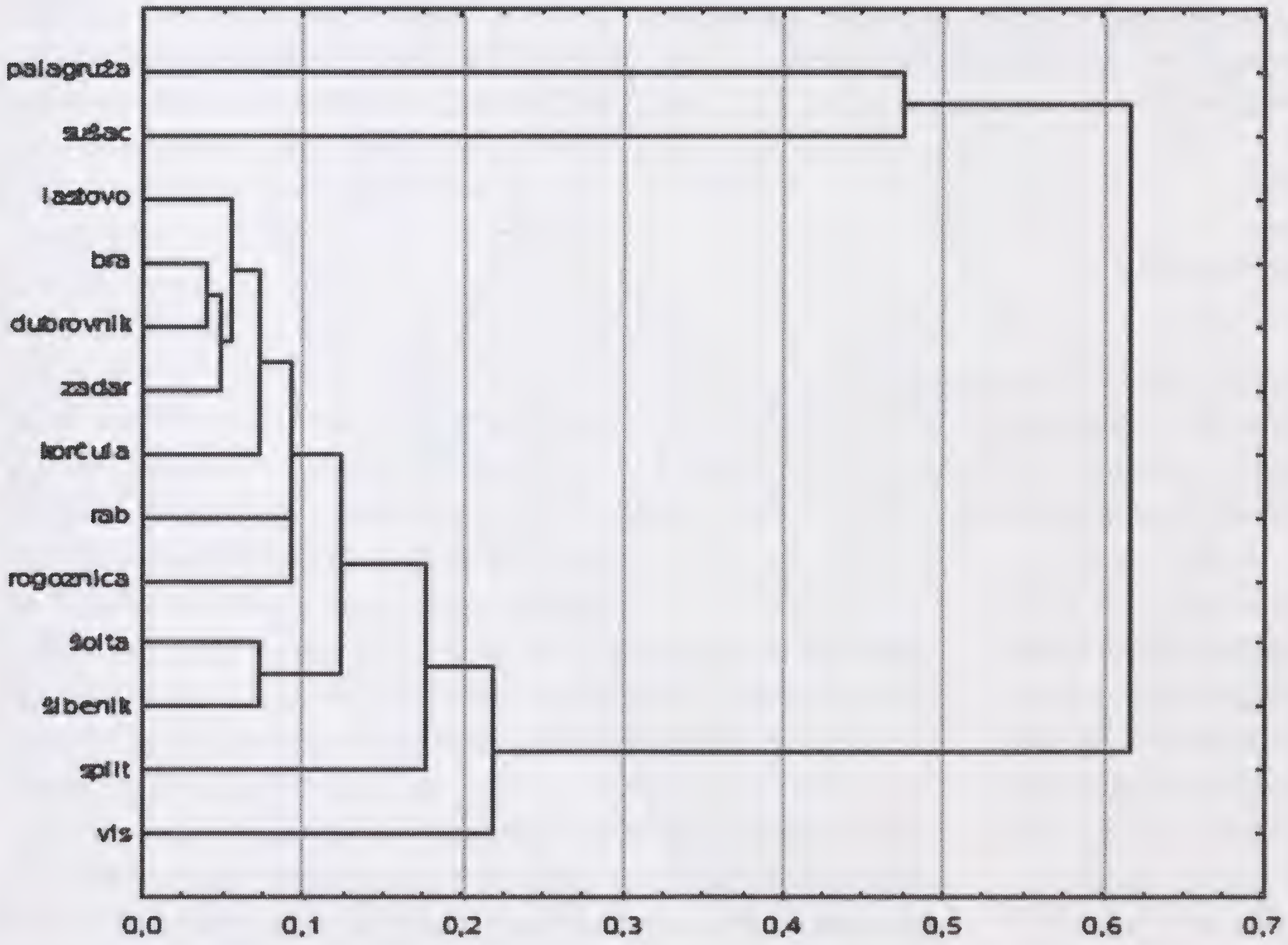

Figure 6. UPGMA cluster analysis: similarity between populations based on three morphometric variables (shell height, shell width, aperture height).

Molecular analysis

Both 16S rDNA and *COI* sequence data showed no significant differences between the South – Adriatic population from the mainland and islands,

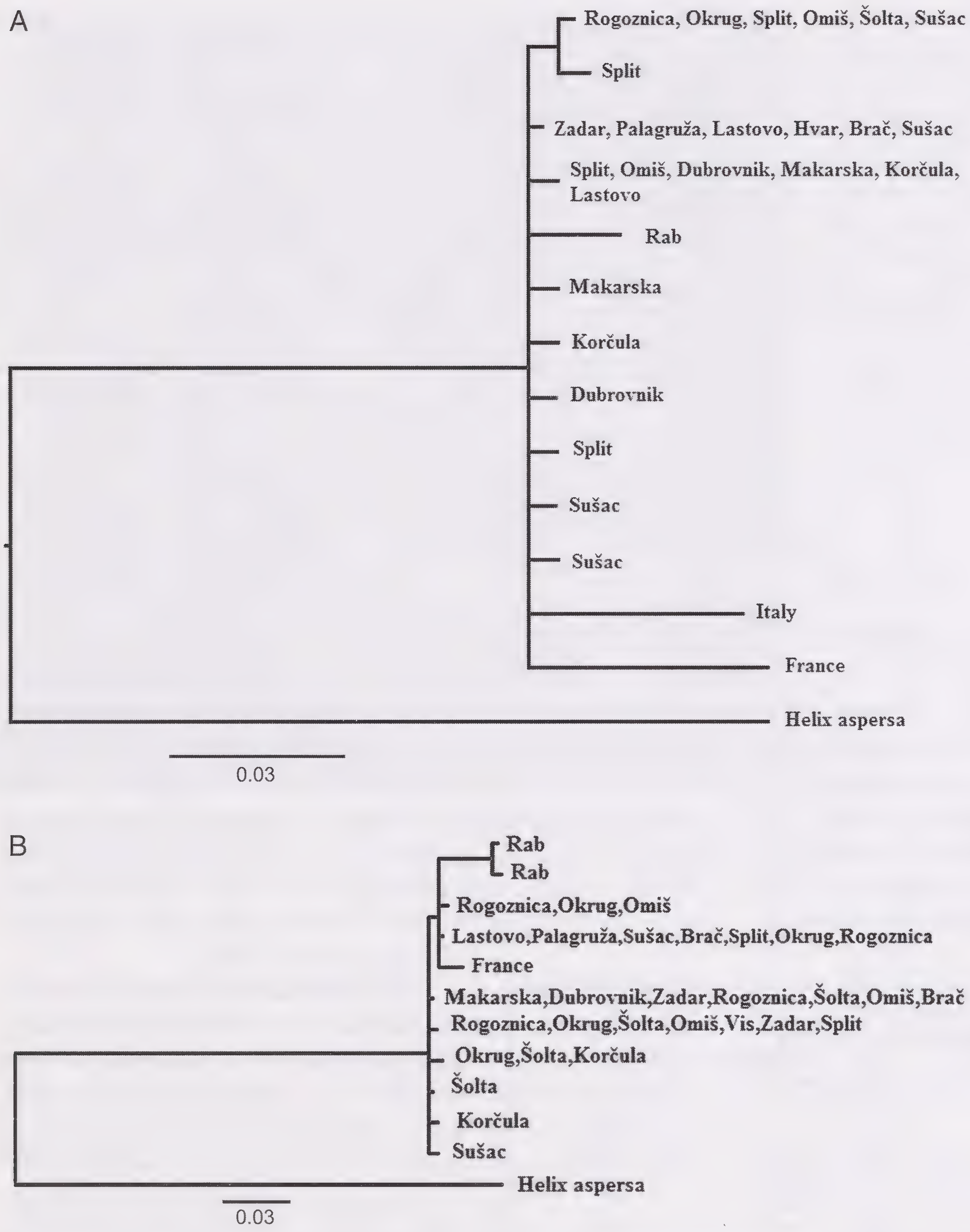
A
Rogoznica, Okrug, Split, Omiš, Šolta, Sušac
Split
Zadar, Palagruža, Lastovo, Hvar, Brač, Sušac
Split, Omiš, Dubrovnik, Makarska, Korčula, Lastovo
Rab
Makarska
Korčula
Dubrovnik
Split
Sušac
Sušac
Italy
France
Helix aspersa
0.03
B
Rab
Rab
Rogoznica,Okrug,Omiš
Lastovo,Palagruža,Sušac,Brač,Split,Okrug,Rogoznica
France
Makarska,Dubrovnik,Zadar,Rogoznica,Šolta,Omiš,Brač
Rogoznica,Okrug,Šolta,Omiš,Vis,Zadar,Split
Okrug,Šolta,Korčula
Šolta
Korčula
Sušac
Helix aspersa
0.03

Figure 7. A, Phylogenetic tree among *Eobania vermiculata* 16S rDNA haplotypes, constructed using Bayesian inference. 16S rDNA sequences from *Helix aspersa* (GenBank AY546357) was used as outgroup. **B**, Phylogenetic tree among *E. vermiculata COI* haplotypes, constructed using Bayesian inference. *COI* sequences from *Helix aspersa* (GenBank AY546277) was used as outgroup.

thus providing no molecular evidences for classification of a single *Eobania vermiculata* species into separate subspecies within investigated area (Figs. 7A and 7B). The UPGMA of concatenated genes (Fig. 8) followed the pattern described above.

DISCUSSION

This study of morphometric characteristics of the shells revealed that populations exposed to anthropogenic activity showed multivariate intrapopulation variation. On the inhabited islands, the people are changing the natural habitat by farming, reforestation and by horticultural activities in populated areas. In this way, ecological conditions of land snails are improved (larger amounts of food and higher humidity) and they show greater variation within populations than individuals that are equally exposed to extreme environmental conditions (Islands of Palagruža and Sušac).

The populations were grouped according to their geographical origin and cluster analyses support that thesis. Populations on open sea islands Palagruža (68 miles S. of Split) and Sušac (25 km W. of the Island of Lastovo) keep their "nanism", as Berberović (1963) stated in his research. The main reason for "nanism" on those islands is geographic isolation (which took place approximately 10,000 years ago) and extreme ecological conditions. Namely, the island of Palagruža belongs to the Csa type climate: Mediterranean climate with hot summers (only 309.7 mm of rain; relative humidity of 74–77%) (Trošić *et al.* 2003). Such climate characteristics are directly reflected on plant communities in which xerophytes are the dominant floral component (Pavletić 1978) and they are not suitable for snail ingestion because of essential oils and silicates in plant tissue. Compared to the starting-morphological variation, our shells showed greater similarity to the sizes found on the islands of Tremiti (Sacchi 1957). Lazaridou *et al.* (1994) stated that the largest shell diameter and the aperture area are negatively related to the mean minimum annual monthly temperatures, and Cook and O'Donald (1971) confirmed that smaller snails survived better in unshaded and warmer conditions. Ecological conditions in the outer islands (limited amount of food, temperature and humidity) could be the reason of increased body size, because those islands are known for extremely unfavorable environmental conditions (mean annual air temperature 16.7° C, 85.3 days per year, minimum air temperature 20° C or higher, annual precipitation 289.5 mm (Trošic´ *et al.* 2003)). Therefore, this climate type known as "Mediterranean arid climate

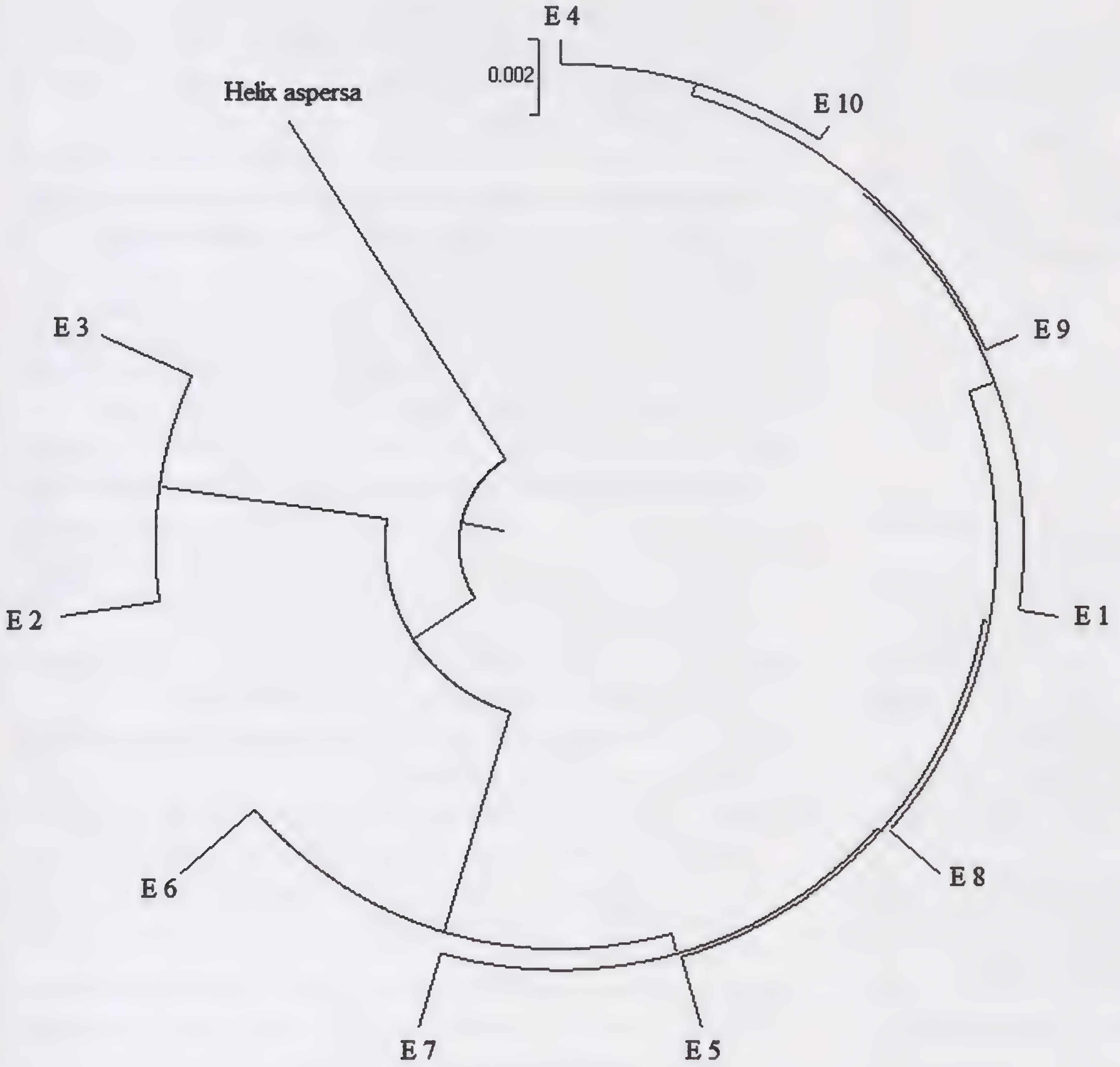

Figure 8. The evolutionary history using the UPGMA method. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide concatenated sequences (E1, Lastovo, Palagruža, Sušac, Brač, Split; E2–E3, Rab; E4, Rogoznica, Okrug, Split, Omiš, Šolta, Sušac; E5, Korčula; E6, Okrug, Šolta, Korčula; E7, Sušac; E8, Šolta; E9, Split, Omiš, Hvar, Dubrovnik, Makarska, Korčula; E10, Split).

type" with a long dry period (more than three months per year), as well as with extremely high temperatures, causes longer hibernation in poikilotherms such as terrestrial snails than in mainland snails. Also, resource availability is reduced. The *Eobania* snails are polyphagous herbivores and the amount of suitable plants (herbs and grasses) is limited (Trinajstić 1973). The islands of Palagruža and Sušac are uninhabited offshore islands app. 68 miles away from the mainland and taking into consideration the formation of these islands it is quite certain that *E.vermiculata* is a native species here.

The populations of inner islands showed greater morphological polymorphism in a way that some individuals reach the standard shell size, almost the same as continental populations. Geographic isolation of those islands is not as long as with the outer ones, and the anthropogenic influence is obvious. The environmental characteristics are quite different from those on the islands of Palagruža and Sušac, because most of the land is agricultural; more water and higher humidity, as well as unlimited supply of food, constitute the main reason why those individuals are bigger than on the islands of Palagruža and Sušac. The populations from the islands of Lastovo and Korčula, according to Berberović (1963), were grouped together withcontinental populations south of the Neretva river. Our survey confirms such shell sizes as mentioned by Berberović (1963), but that group also includes the populations from the island of Brač. The continental populations from that group are populations from Zadar and Dubrovnik.

The continental populations north of the Cetina river are similar to the populations from the islands of Rab and Šolta, which is in accordance with Bereberović (1963). The continental populations from the area between the rivers Cetina and Neretva are in the same group with populations from Split and island of Vis. According Berberović (1963), the populations from the Island of Vis are defined as a mixed zone of different types of shell sizes. Our survey confirms this position. Our survey did not confirm a reduction in shell size towards Berberović's (1963) reduction, continent-inner islands-outer islands. We found an enlargment of shell sizes, with the exception of the outer islands, probably caused by anthropogenic influence and changes in environmental conditions.

Both 16S phylogenetic tree and haplotype network revealed a lack of genetic differentiation among investigated specimens, and no distinct lineages could be identified. Only north Adriatic population from the island of Rab formed a separate but closely related clade. *COI* sequences were more variable, and revealed a separate position of Rab specimens in both phylogenetic trees and haplotype network. This result will be explained in another phylogenetic study currently being prepared for publication.

Biogeography shows intermixed distribution of 16S and *COI* haplotypes along this area and argues against the classification of a single *Eobania vermiculata* species into separate subspecies. Molecular analysis of two mitochondrial genes (Puizina *et al.* GenBank unpublished sequences) did not support subspecific status of *E. vermiculata pelagosana* on the molecular level, so those samples could be defined as ecotypes or forms. Molecular analysis of 16S rDNA and *COI* (Puizina *et al.*, unpublished sequences GenBank accession numbers JF277380–JF277396 and JF802030–JF802030) showed that there is no molecular evidence for a classification of a single *E. vermiculata* species into separate subspecies within the investigated area.

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Synonymy of *Sonorella rosemontensis* Pilsbry, 1939 with *Sonorella walkeri walkeri* Pilsbry and Ferriss, 1915 (Pulmonata: Helminthoglyptidae) from the Santa Rita Mountains, Arizona, U.S.A.

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Abstract: In the northern Santa Rita Mountains, Pima County, Arizona, there are three named species of the helminthoglyptid landsnail *Sonorella* Pilsbry, 1900: *Sonorella magdalenensis* (Stearns, 1890), *S. walkeri walkeri* Pilsbry and Ferris, 1915, and *S. rosemontensis* Pilsbry, 1939. Of these taxa, Pilsbry (1939) originally named *S. rosemontensis* based on shell characters alone. Later, he dissected and described the reproductive organs from a specimen preserved in alcohol that was collected at a location different from the type locality. Subsequently, Walter B. Miller collected two snails, calling them *S. rosemontensis,* because they were collected at Pilsbry's *S. rosemontensis* locality. He noted that their male genitalia did not resemble those described by Pilsbry; rather, they closely resembled those of *S. w. walkeri*. Our examination of shell and reproductive anatomies of snails from the *S. rosemontensis* type locality near the north end of the Santa Rita Mountains, as well as specimens in the Santa Barbara Museum of Natural History collection lead us to conclude that snails previously referred to as *S. rosemontensis* are synonymous with *S. w. walkeri*, and that in his original description of the reproductive anatomy of *S. rosemontensis,* Pilsbry mistakenly dissected a specimen of *S. magdalenensis*.

Key words: talus snails, land snail, Santa Rita Mountains, verge, anatomy

Sonorella rosemontensis H. A. Pilsbry, 1939 was described in error. The reproductive anatomy, as drawn by Pilsbry (1939), does not match the anatomy of snails subsequently collected from the type locality and no other collections of snails that we are aware of contain specimens resembling Pilsbry's description. The correct depiction of the reproductive tract of a snail collected from the *S. rosemontensis* type locality appears on page 260 of Walter B. Miller's doctoral dissertation (Miller 1967). Miller's drawing of the reproductive tract of *S. rosemontensis* does not appear to differ in any significant way from that of *S. walkeri walkeri* Pilsbry and Ferriss, 1915 (Miller 1967: 260).

Pilsbry (1939) named *Sonorella rosemontensis* based on a shell that he originally described as a range extension of *S. hesterna* Pilsbry and Ferriss, 1923 (Pilsbry and Ferriss 1923, Bequaert and Miller 1973). The internal anatomy was disregarded as an identifying characteristic since *S. hesterna* was known only from shells at its original locality. Shell size and embryonic sculpture seemed the same as those of *S. hesterna* in the nearby Rincon Mountains.

Later, Pilsbry (1939) seems to have mistakenly dissected the genitalia from a different species of *Sonorella*, which he describes as being, "very closely related to *S. arida* Pilsbry and Ferriss, 1939, the genitalia being of the same general character…". *Sonorella arida*, *S. tumamocensis* Pilsbry and Ferriss, 1915, and *S. linearis* Pilsbry and Ferriss, 1923 were synonymized with *Sonorella magdalenensis* (Stearns, 1890) by Miller (1967). Pilsbry apparently confused the shell of the specimen he dissected with that of *S. hesterna*, which are very similar to the shells of *S. walkeri,* as they are in the same *Sonorella* complex (*S. hachitana* Dall, 1985) (Bequaert and Miller 1973). Furthermore, Pilsbry (1939: 349) went on to say, "It [*Sonorella rosemontensis*] was formerly considered to be identical with *S. hesterna*, but the well-developed threads of the embryonic shell apparently indicate a different species. Were it not for the very different verge, this form would hardly be separated from *S. walkeri*." Thus, Pilsbry was well aware of the distinct features of the animal he dissected, which resembled two different known species.

This disparity in reproductive structures of the specimens that Walter B. Miller collected at the type locality of *Sonorella rosemontensis*, compared to the description and drawings in Pilsbry (1939) was first noted by Miller (Miller 1967: 70). He states: "The genitalia … are not at all those of the [*Sonorella*] *tumamocensis* group [*i.e., Sonorella magdalenensis*]. Instead, they resemble those of *S. walkeri*. The penis is very short and thin as is [*Sonorella*] *walkeri*…". Miller goes on to say that: "It is probable that he [Pilsbry] dissected a

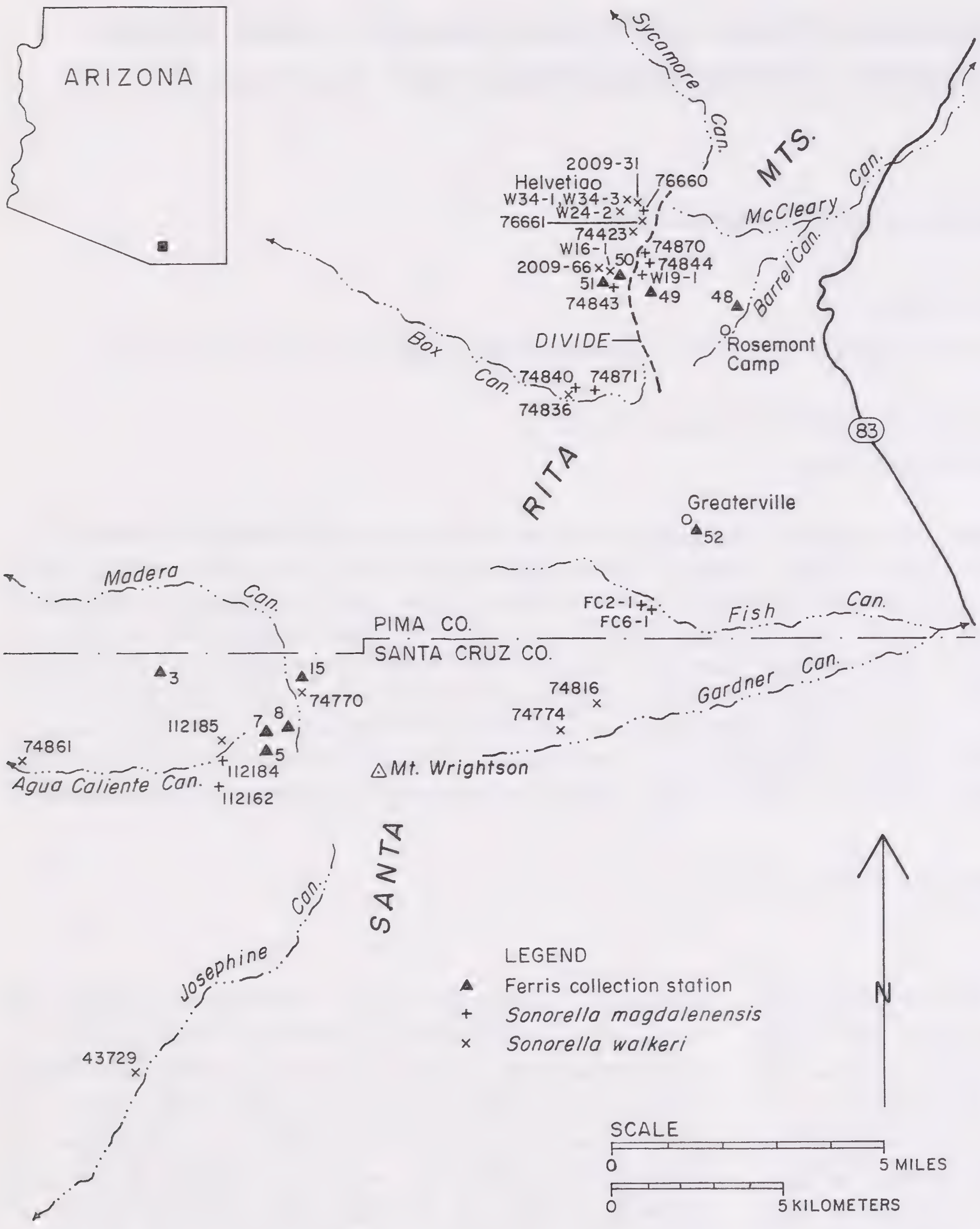

Figure 1. Map of collection sites of *Sonorella walkeri* (= *S. rosemontensis*) and *S. magdalenensis* in the Santa Rita Mountains. Numbers beside symbols indicate museum numbers, WestLand collection numbers, or Ferriss' stations.

specimen of *S. tumamocensis linearis* by mistake. I have dissected an adult specimen of [*S.*] *rosemontensis* and find the genitalia to be as described above… *S. rosemontensis* is closely related in all respects to *S. walkeri*."

Pilsbry (1939) did not describe the collection locality of *Sonorella rosemontensis* in any greater detail than: "Northern end of the Santa Rita Mountains near Rosemont." However, examination of the collection localities for *S. hesterna* in the Santa Rita Mountains, show that snails were collected from "above … Rosemont Camp" (Ferris' Station 48), at a saddle overlooking Helvetia (Station 50), and on a talus slope south of Greaterville, Arizona (Station 52; Ferriss' Collection Notes 1917-18; Pilsbry and Ferriss, 1923). Examination of collections at The Academy of Natural Sciences of Philadelphia (ANSP) showed that the holotype of *S. rosemontensis* was collected at Ferriss' station 49, on the east side of the divide, and consists of a single shell (ANSP 166642). Pilsbry then dissected a snail from station 50, on the west side of the divide, calling it the paratype (ANSP 118058). This paratype contains a dried up alcoholic specimen, and station 50 is the only station in the north end of the Santa Rita Mountains where Ferriss collected live snails (Ferriss' collection notes 1917-18). This evidence strongly suggests that Pilsbry used the shell collected at one location and the reproductive anatomy from another to describe *S. rosemontensis.*

In Miller (1967) and Bequaert and Miller (1973) *Sonorella walkeri* and *S. rosemontensis* are listed as separate species, though they are described as being very similar. In a later report to the U. S. Fish and Wildlife Service on invertebrates of the Rosemont area, Miller (1978) wrote: "Careful examination of shells and reproductive anatomies revealed no significant difference [of *S. rosemontensis*] from *S. walkeri* Pilsbry and Ferriss, which is common further south…" He went on to say "It is the opinion of the author that *S. rosemontensis* is at least conspecific with *S. walkeri* and may possibly be a synonym."

The purpose of this paper is to present the results of an analysis of specimens from Miller's collection of the reproductive anatomies of *Sonorella* species collected in the type location of *Sonorella rosemontensis* and to present an analysis of the reproductive anatomies of specimens recently collected at the same location. Together, these analyses provide evidence that *S. rosemontensis* was named in error and is synonymous with *Sonorella walkeri.*

MATERIALS AND METHODS

Species considered in this study are: *Sonorella magdalenensis* (Stearns, 1890); *S. walkeri walkeri* Pilsbry and Ferriss,

Table 1. Lengths of measurements of the genital organs of *Sonorella rosemontensis*, *S. walkeri*, and *S. magdalenensis* (= *S. tumamocensis*), Walter B. Miller Collection, Santa Barbara Museum of Natural History and results of measurements taken on slides of reproductive tracts of *Sonorella* spp. we collected at the north end of the Santa Rita Mountains to the nearest 0.1 mm.

Number	Species name	Verge (mm)	Vagina (mm)	Penis (mm)
A. Walter B. Miller Collection				
74923A	*S. rosemontensis*	1.3	5.1	2.7
76661	*S. rosemontensis*	2.9	9.4	3.9
	Mean (*N*)	2.1 (2)	7.2 (2)	3.3 (2)
	Range	1.3–2.9	1.5–9.4	2.7–3.9
B. Walter B. Miller Collection				
74770	*S. walkeri*	1.2	8.3	3.3
74774B	*S. walkeri*	3.3	1.2	4.4
74795B	*S. walkeri*	2.0	5.3	3.1
74795C	*S. walkeri*	-	-	4.0
4795D	*S. walkeri*	2.4	9.9	3.9
74876C	*S. walkeri*	-	-	4.0
77219	*S. walkeri*	2.8	9.9	3.4
74876D	*S. walkeri*	2.0	-	3.4
74876E	*S. walkeri*	2.0	4.5	4.5
74876F	*S. walkeri*	2.3	-	4.3
	Mean (*N*)	2.2 (8)	8.3 (6)	3.8 (10)
	Range	1.2–3.3	1.2–9.9	3.1–4.5
C. WestLand Collection				
W34-1	*S. walkeri*	1.2	5.8	3.2
W24-2	*S. walkeri*	1.5	7.5	3.2
W23-4	*S. walkeri*	1.3	9.5	2.0
W34-3	*S. walkeri*	1.5	-	2.8
FC2-1	*S. walkeri*	1.2	-	-
	Mean (*N*)	1.3 (5)	7.6 (3)	2.8 (4)
	Range	1.2–1.5	5.8–9.5	2.0–3.2
D. Walter B. Miller Collection				
74739	*S. tumamocensis*	4.1	9.3	10.2
74742(3)	*S. tumamocensis*	4.3	3.5	10.4
74742 (2)	*S. tumamocensis*	4.4	-	10.2
74742	*S. tumamocensis*	4.7	15.3	9.8
77453H	*S. magdalenensis*	6.4	17.9	14.7
	Mean (*N*)	4.8 (5)	11.5 (4)	11.1 (5)
	Range	4.1–6.4	3.5–17.9	9.8–14.7
E. WestLand Collection				
E12-1	*S. magdalenensis*	10.5	7.5	19.0

1915; and *S. rosemontensis* Pilsbry, 1939 (Pulmonata: Helminthoglyptidae) from the Santa Rita Mountains, Arizona.

Material for this investigation came from two sources. First, *Sonorella* spp. were collected in 2008, 2009, and 2011. The habitat for *Sonorella* in this locality consists of talus slopes that occur on both the east and west sides of the north-south oriented divide of the Santa Rita Mountains just east of Helvetia, Arizona. Specimens in 2008 and 2009 were collected from the northern Santa Rita Mountains in the vicinity of Helvetia (Fig. 1), the locality of the *Sonorella rosemontensis* type specimen. Additionally, eight snails were collected in 2011; four from the west side of the divide and one from the east side of the divide and three from the mountains near Fish Creek (Fig. 1). Second, we studied material that was part of Miller's mounted reproductive tracts and shells of *S. rosemontensis*, *S. walkeri*, and *S. magdalenensis*; part of the Walter B. Miller Collection at the Santa Barbara Museum of Natural History (SBMNH), Santa Barbara, California.

Eight living snails were drowned and their bodies were removed from their shells. Reproductive organs were dissected free of the other organs, stained with Delafield Hematoxylin and Eosin B and mounted on slides in the manner described by Gregg (1959) and later revised by Naranjo-García (1989). Reproductive tracts from snails were measured using an ocular micrometer under a dissecting microscope at 40X and scaled to millimeters.

We dissected several specimens of *Sonorella walkeri* and *S. magdalenensis* collected by personnel from WestLand in 2008 and 2009 that were preserved in 70% ethyl alcohol. These specimens were mounted and examined to provide a comparison of their shape to other specimens. Preservation in alcohol prior to dissection results in dehydration, which causes substantial shrinkage in length of tissues (5–40%: Fowler and Smith 1983, Radtke 1989, Jennings 1991), thus we did not use these specimens for tissue measurements.

All of the slide-mounted reproductive tracts of *Sonorella* species collected from the Santa Rita Mountains in the Walter B. Miller Collection at the SBMNH were scanned adjacent to

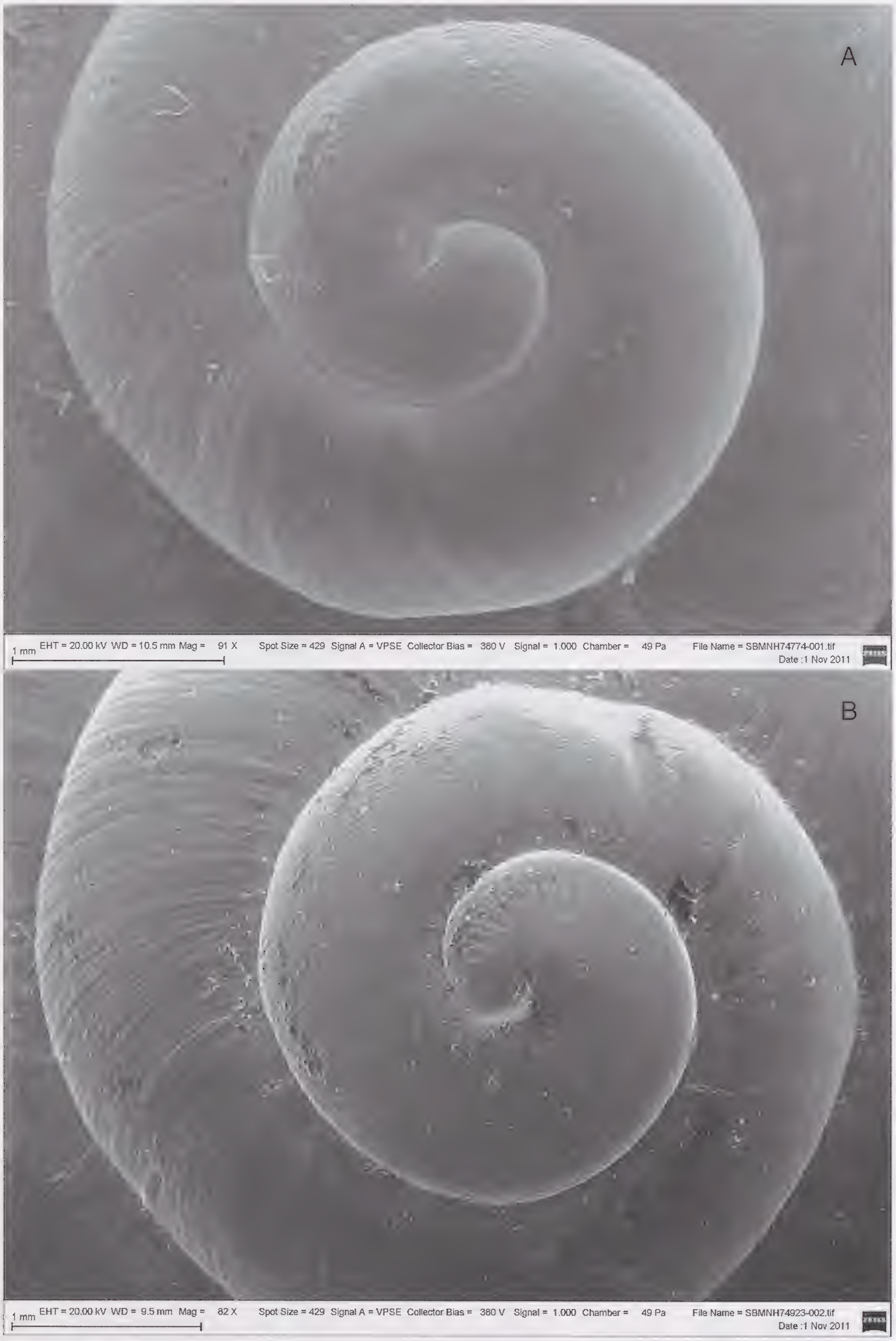
A
1 mm
EHT = 20.00 kV WD = 10.5 mm Mag = 91 X
Spot Size = 429 Signal A = VPSE Collector Bias = 380 V Signal = 1.000 Chamber = 49 Pa
File Name = SBMNH74774-001.tif
Date :1 Nov 2011
B
1 mm
EHT = 20.00 kV WD = 9.5 mm Mag = 82 X
Spot Size = 429 Signal A = VPSE Collector Bias = 380 V Signal = 1.000 Chamber = 49 Pa
File Name = SBMNH74923-002.tif
Date :1 Nov 2011

Figure 2. Scanning electron micrograph images of the shells of: **A**) *Sonorella walkeri* (SBMNH 74774) and **B**) *S. rosemontensis* (SBMNH 74923) showing the sculpturing of the embryonic shell.

a millimeter scale. The digital images of the reproductive tracts were examined at the maximum size in which both the reproductive tract and the millimeter scale could be measured from the screen image and lengths of the reproductive structures were scaled to determine actual length in millimeters. At the same time, numerous shells were photographed, including those of the scanned specimens. In addition, scanning electron micrographs (SEMs), using a Zeiss EVO40 XVP SEM, were taken of the embryonic whorls of a specimen of *Sonorella rosemontensis* and a *S. walkeri* from Miller's collection (SBMNH).

RESULTS

Identification of snails

Two species of *Sonorella* were identified from our mounted specimens: *Sonorella walkeri* and *S. magdalenensis*. The specimens of *S. walkeri* (five mature fresh dissected, two immature fresh dissected, and two alcohol-preserved specimens) had thin verges that narrowed towards the tip with a subterminal pore. Three specimens (one mature fresh dissected and two alcohol preserved) were identified as *S. magdalenensis* based on the larger, spirally plicate and cone-tipped verge (for an illustration of the genitalia of *S. walkeri,* see Pilsbry 1939: 287, fig. 157; for *S. magdalenensis* (as *S. arida)* see p. 343, fig. 211).

Range

The collection locations of *Sonorella walkeri* and *S. rosemontensis* are overlapping and continuous throughout the Santa Rita Mountains (Fig. 1). Pilsbry's and Miller's collections of *S. walkeri* are located in the Santa Rita Mountains as far north as Greaterville, Arizona. Ferris collected snails, identified from shells by Pilsbry as *S. rosemontensis,* in Greaterville (ANSP 166642, 118058, 166641, 166639). Snails we collected and identified as *S. walkeri* were found located on both sides of the divide near Helvetia and Rosemont and from Fish Creek near Greaterville. There is no evidence of allopatric ranges of these two species. The range of *S. magdalenensis* is also widespread through the Santa Rita Mountains and is sympatric with the range of *S. walkeri* (Fig. 1).

Genitalia

The shape of the verge of *Sonorella walkeri* as illustrated by Pilsbry (1915), Miller (1967), and seen in our specimens is identical to the shape of the verge of the *S. rosemontensis* specimens collected by Miller (1967) and as seen in the examination of his preserved specimens.

The verge of all of these specimens is thin with a pointed tip and possesses a subterminal pore. The verge of these specimens is entirely different from the robust verge described by Pilsbry (1939) for *S. rosemontensis*, which is spirally plicate with a cone-shaped tip, a trait of *S. magdalenensis*. We are not aware of any specimens collected since Pilsbry (1939) named *S. rosemontensis* that have a verge of the shape described therein.

The measurements of the reproductive tracts of *Sonorella walkeri* and *S. rosemontensis* collected and mounted by Miller and those we collected and mounted have completely overlapping ranges of sizes (Table 1). The verges of Miller's *S. walkeri* range in size from 1.2–3.3 mm (nearly a 3-fold difference between the smallest and the largest specimens) and those of *S. rosemontensis* range from 1.3–2.9 mm. The lengths of the verge of the five specimens we collected range in size from 1.2–1.5 mm, within the range of Miller's *S. walkeri* specimens. The penis lengths are variable but not as variable as the verge (Table 1). The vagina lengths are much more variable with a nearly six-fold difference (Table 1). There are notable differences in the size of the verge, penis, and vagina and in shell size in the two specimens of *S. rosemontensis* (SBMNH 74923 and 76661) collected by W. B. Miller in very similar, nearby locations about 10 years apart (Table 1).

Sonorella magdalenensis has a male anatomy distinctly different from *S. walkeri* and *S. rosemontensis* in Miller's collection and in our specimens. Its verge is more than twice as long on average (Table 1D, 1E), has a cone-shaped tip, and is spirally plicate along its length. We collected three specimens of *S. magdalenensis*. Of the three, one (E12-1), was relaxed and counterstained. It had a verge length of 10.5 mm (Table 1E).

Embryonic shell sculpturing

Comparison of the scanning electron micrographs of the embryonic whorls of a *Sonorella walkeri* (Fig. 2a) and *S. rosemontensis* (Fig. 2b) show the same features – punctations and spiral lines. Thus, there is no indication that the morphology of the embryonic whorls indicates that these individuals belong to different species.

DISCUSSION

In 1939, Pilsbry stated of the genus *Sonorella*: "The male genitalia have been much more modified [as opposed to the shells], and the classification of the genus is therefore based upon these organs." Pilsbry 1939: 270). In this paper, we have examined the reproductive tracts of *Sonorella walkeri* and *S. rosemontensis* collected by W. B. Miller and the reproductive tracts that we collected and mounted. There are no discernible differences in the shapes or sizes of the male or female reproductive organs among these specimens. We also examined the embryonic whorls of *S. walkeri* and *S. rosemontensis* and found no discernible differences in the microscopic sculpturing of these shells. Based on these morphological data and the sympatric range of *S. walkeri* and *S. rosemontensis*, we conclude that the names of these species are synonymous. Because *S. walkeri* Pilsbry and Ferris, 1915 has precedence, this name is valid and the name *S. rosemontensis* is a junior objective synonym and therefore is a *nomen nudum* (Mayr 1969).

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We dedicate this paper to Walter B. Miller who was the mentor of JEH and LHG and whose work on *Sonorella* in the southwestern United States has greatly expanded our understanding of the taxonomy and range of these interesting snails. We are very grateful to Paul Valentich-Scott, Curator of Malacology at the Santa Barbara Museum of Natural History, for the invitation to visit the museum and for providing generous assistance to us during our stay. We thank the editor and two anonymous reviewers for numerous helpful suggestions. Funding for this work was provided by Rosemont Copper Company by contract to WestLand Resources, Inc.

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Report on the Terrestrial Mollusks of the Sierra de la Madera (Oposura), Sonora, Mexico – The Caracoleros

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Abstract. The Madrean Archipelago Biological Assessment (MABA) "bioblitz" expedition to Sierra de la Madera (Oposura) near Moctezuma, Sonora, Mexico from 29 July–7 August 2010 included a land snail inventory. Five people searched for both macro- and microsnails in five sites in foothills thornscrub, oak woodland, and pine-oak forest (1225–2080 m elevation) and found 347 individuals of 18 species or forms. Macrosnails included a *Sonorella* Pilsbry, 1900 species ($N = 5$) found at four sites, a succineid species ($N = 3$) from two sites, and one philomycid slug. Micromollusks (< 6 mm) included representatives of seven families. The vertiginid genus *Gastrocopta* Wollaston, 1878 included eight species/forms. *Vallonia perspectiva* Sterki, 1892 was the most abundant species ($N = 123$). This collection adds four species to the 59 previously reported for the state. Seven forms from this southern "sky island", including the *Gastrocopta ashmuni* group (=*Immersidens ashmuni* Pilsbry and Vanatta, 1900) and *Sonorella*, are characteristic of the Southwestern Mollusk Province (SWMP). Collecting land snails in Sonora has been sporadic because of the difficult terrain and remote locations. Additional efforts and locations should add many more species and clarify biogeographical affinities of this rugged area.

Resumen. El programa de la Evaluación de la Biodiversidad del Archipiélago Madrense organize una expedición a la Sierra de la Madera (Opusura) cerca de Moctezuma, Sonora, Mexico, para realizar un muestreo biológico intensivo (bioblitz) del 29 julio hasta al 7 agosto 2010, el cual incluyó un inventario de caracoles terrestres. Cinco personas buscaron tanto macro como micromoluscos en cinco sitios en matorrales espinosos, bosques de encino y de pino-encino (1225–2080 m de elevación) y encontraron 347 individuos de 18 especies o formas. Los macromoluscos incluyen una especie de *Sonorella* Pilsbry, 1900 ($N = 5$) encontrada en cuatro sitios, una especie de *Succinea* Draparnaud, 1801 ($N = 3$) de dos sitios, y una babosa de la familia Philomycidae. Los micromoluscos (< 6 mm) incluyen representantes de siete familias. El genero *Gastrocopta* Wollaston,1878 de la familia Vertiginidae incluye ocho especies/formas. *Vallonia perspectiva* Sterki, 1892 fue la especie más abundante ($N = 123$). Esta colecta añade cuartro especies a las 59 previamente reportadas para el estado. Siete formas de estea "isla serrana" del sur entre ellos el grupo *Gastrocopta ashmuni* Sterki, 1896 y *Sonorella* Pilsbry,1900, son caracteristicas de la Provincia Malacológica del Suroeste. La recolección de caracoles terrestres en Sonora ha sido esporádica debido a las dificultades del terreno y lugares remotos. El trabajo de campo en áreas nuevas añadirá muchas más especies y aclarará las afinidades biogeográficas de esta zona escarpada.

Key words: Mexican land snails, sky islands, MABA, biodiversity

The third Madrean Archipelago Biological Assessment (MABA) expedition into northeastern Sonora in July and August 2010 was a "bioblitz" sponsored by the Sky Island Alliance as part of its mission to survey, protect and restore unique habitats of the mountain islands and desert seas of the United States/Mexico borderlands. Participants included nearly 30 science professionals and students from both Mexico and the United States with a wide range of expertise. Five people, "los caracoleros", worked on land snails. The target of the bioblitz was the Sierra de la Madera (Oposura), an isolated granitic-batholithic mountain range covering about 800 km^2 in the greater Río Yaqui drainage in northeastern Sonora near the town of Moctezuma. The mountain included foothills thornscrub vegetation around its base as well as more mesic habitats grading into pine–oak forest above 1700 m. Some higher elevations of the Sierra de la Madera are part of the Reserva Forestal Nacional y Refugio de Fauna Silvestre Ajos-Bavispe in the Comisión Nacional de Áreas Protegidas (CONANP) system (T. R. Van Devender pers. comm. 2010).

MATERIALS AND METHODS

Land snails were sampled at five sites in the Sierra de la Madera (within the municipios or "counties" of Cumpas and Moctezuma) in northwestern Sonora, Mexico (Figure 1).

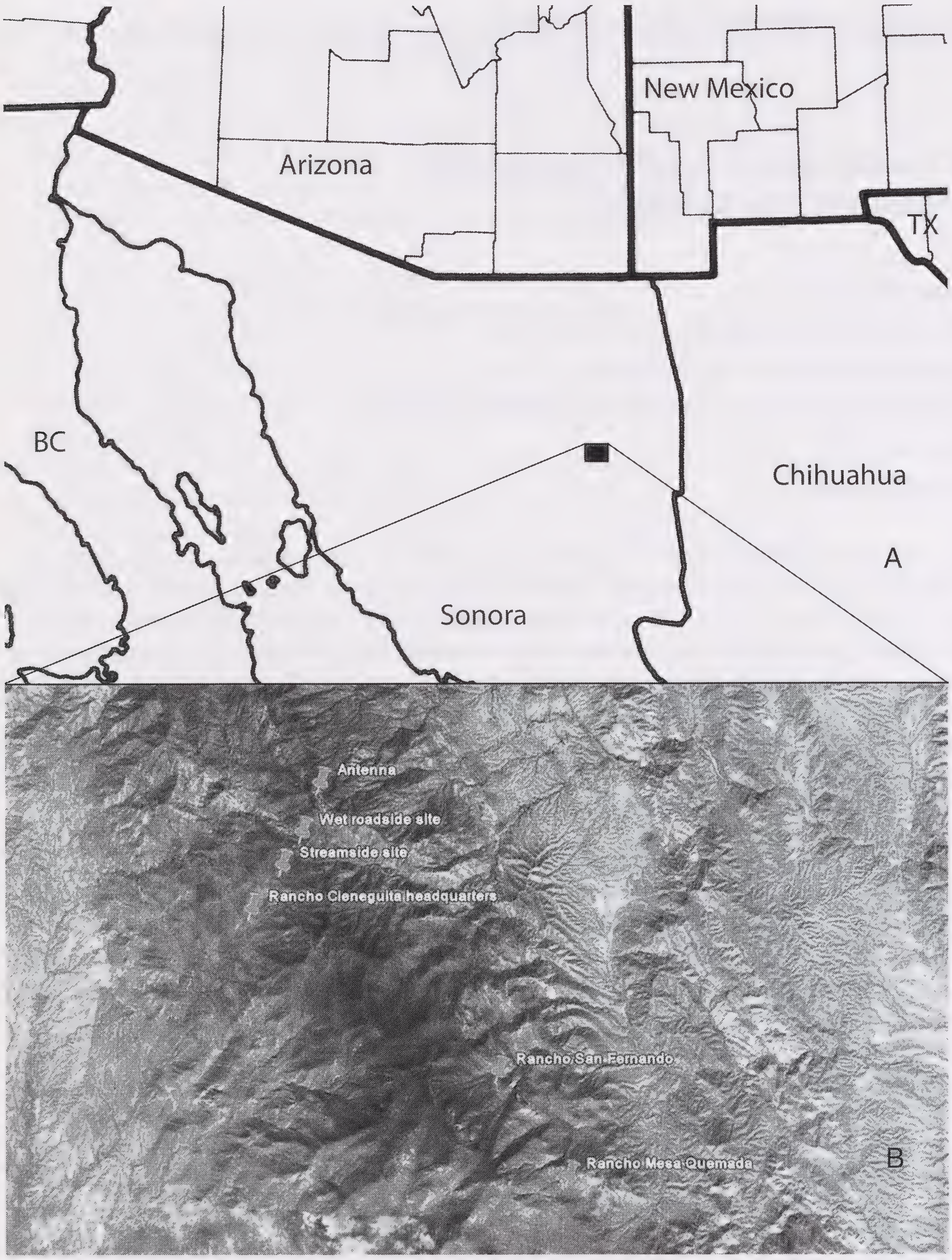

Figure 1. Collection locations. **A.** Northern Mexico and southwestern United States with location of Sierra de la Madera. **B.** Location of sites visited (Google Earth projection July 2011).

Elevation, orientation and descriptions were recorded at each site. Likely microhabitats under rocks, downed wood, and leaf litter were searched visually. Samples of leaf litter, humus and soil were collected at four sites, sundried, sifted through sieves (0.855–3.35 mm mesh) and searched using hand lenses. Live snails were drowned in tap water laced with menthol crystals and preserved in 70% EtOH. Most specimens were brought back to the U.S. under SEMARNAT permit #055 to Dr. Edna Naranjo-García, but one slug and two succineids were transported to Mexico City. Most specimens will be deposited into the Colección Nacional de Moluscos in the Instituto de Biología, Universidad Nacional Autónoma de México in Mexico City. Specimens were cleaned and imaged using cameras, microscopes, and scanning electron microscopes (SEM). Data recorded for most intact specimens were number of whorls (WH), diameter (D) and height (H) as measured to ± 0.01 mm from micrographs using analysis in Adobe Photoshop™. Width of umbilical opening (U) was measured for many specimens. Samples were compared using the conservative two-tailed T-tests with unequal variance (Steele and Torrie 1980) using Microsoft Excel™.

Specimens were identified using figures, keys, and descriptions in Pilsbry (1940, 1946, 1948), Bequaert and Miller (1973), Naranjo-García and Fahy (2010), and Nekola and Coles (2010). Fred Thompson (University of Florida), Jeff Nekola (University of New Mexico), Barry Roth (San Francisco, California), and Harry G. Lee (Jacksonville, Florida) were consulted for opinions on problematic specimens. Taxonomy of Bouchet and Rocroi (2005) was used. Information on the biogeography, botany, and geology of the region was obtained from T. R. Van Devender.

RESULTS

Sites

Three sites were on the north or northwest part of the mountain range in Municipio de Cumpas, Sonora (Figure 1). Collection site 1 was a steep, north facing, mesic canyon at 1584 m elevation above Rancho Cieneguitas, (29.9948°N, 109.5593°W). Four people searched this site for approximately four hours (16 person-hours). Most snails were in litter collected on the steep slope above the canyon floor. Site 2 was in pine-oak woodland near a microwave antenna at 2080 m elevation (30.0196°N, 109.5457°W). Snails were found under bark on logs, in talus slides, and in leaf litter during about two hours of searching (8 person-hours). Site 3 was a steep north-facing road cut at 1832 m elevation (30.0029°N, 109.5538°W). Water seeping from crevices and ferns among the rocks indicated a particularly moist area. The site was searched for about 3 person-hours. Remaining sites were on the southeastern flank of the range in Municipio de Moctezuma, Sonora (Fig. 1). Site 4 included roadside rocks, a small arroyo, and part of a wooded canyon at about 1225 m elevation on Rancho Mesa Quemada (29.8952°N, 109.4573W). About 10 person-hours were spent searching this site. Site 5 included edges of a large meadow and a semi-permanent stream in a steep canyon at Rancho San Fernando (29.8952°N, 109.4826°WW) near 1490 m elevation. Snails were found in litter under logs, under bark on dead trees, on wet rock walls and in the riparian zone. Searches at this site involved more than 20 person-hours of effort. Additional images and maps are available in the MABA database <http://www.madrean.org/maba/symbfauna/imagelib/index.php>.

Snails collected

A total of 347 live animals or identifiable shells were collected. Only nine individuals of two snail and one slug species were larger than 5 mm. Nearly all other snails were extracted from soil/litter samples. Snails represented 10 families, 11 genera and 18 species or forms listed below and in Table 1. Identification of most species was hampered by lack of comparative material and high quality illustrations of whole animals, shells, or aperture lamellae for similar taxa. We provide these illustrations for most taxa encountered. Snails were distributed heterogeneously among sites. Eleven species were found at only one site, and five more species were found at only two sites. The remaining two species were found at three or four sites. No species was found at all sites. The only "invasive" species was *Zonitoides arboreus* (Say, 1816), which was fairly rare at a disturbed site. Snail diversity varied considerably among sites at Sierra de la Madera; but only site one had sufficient numbers to provide a reliable estimate of diversity (Cameron and Pokrysko 2005). Both the 13 species and Shannon-Wiener diversity estimate ($h' = 1.674$) were much lower at this site than we would expect in similar sized samples from eastern U.S.A.

Family Gastrodontidae

One perfect shell with the distinctive ribbing of *Striatura meridionalis* (Pilsbry and Ferriss, 1906) (Fig. 2D–F) was found at Rancho San Fernando (Site 5). It has 3 whorls, D = 1.53 mm, H = 0.49 mm, and U = 0.32D. The first 1.25 whorls have conspicuous incised lines but no riblets. Ten major riblets are equally spaced in 0.49 mm of the last whorl. This shell is the first record of the species from Sonora. *S. meridionalis* is widespread in eastern U. S. and ranges to Texas, New Mexico, and Arizona (Bequaert and Miller 1973, Hubricht 1985, Metcalf and Smartt 1997). *Striatura meridionalis* also occurs

Table 1. Snails collected at each site at Sierra de la Madera, Sonora, Mexico.

		Sites					
Family	Species	1	2	3	4	5	All
Gastrodontidae	*Striatura meridionalis*					1	1
Gastrodontidae	*Zonitoides arboreus*		3				3
Helicarionidae	*Habroconus trochulinus*					12	12
Helminthoglyptidae	*Sonorella* species	1	1	1		2	5
Philomycidae	*Pallifera arizonensis*	1					1
Punctidae	*Punctum* species	32					32
Vertiginidae	*Gastrocopta ashmuni*	16				8	24
Vertiginidae	*Gastrocopta cochisensis*	4					4
Vertiginidae	*Gastrocopta dalliana*				6		6
Vertiginidae	*Gastrocopta* cf. *dalliana*	38				8	46
Vertiginidae	*Gastrocopta pellucida*	4					4
Vertiginidae	*Gastrocopta perversa*	3					3
Vertiginidae	*Gastrocopta perversa* form *sana*				9	9	
Vertiginidae	*Gastrocopta pilsbryana*	2					2
Succineidae	*Succinea* species	1				2	3
Thysanophoridae	*Thysanophora horni*	6			17	1	24
Valloniidae	*Vallonia perspectiva*	120			3	123	
Zonitidae	*Glyphyalinia* cf. *umbilicata*	23	1		2	19	53
	Total	251	5	1	37	53	347
	Total species	13	3	1	5	8	18
	Unique species	6	1	0	2	2	

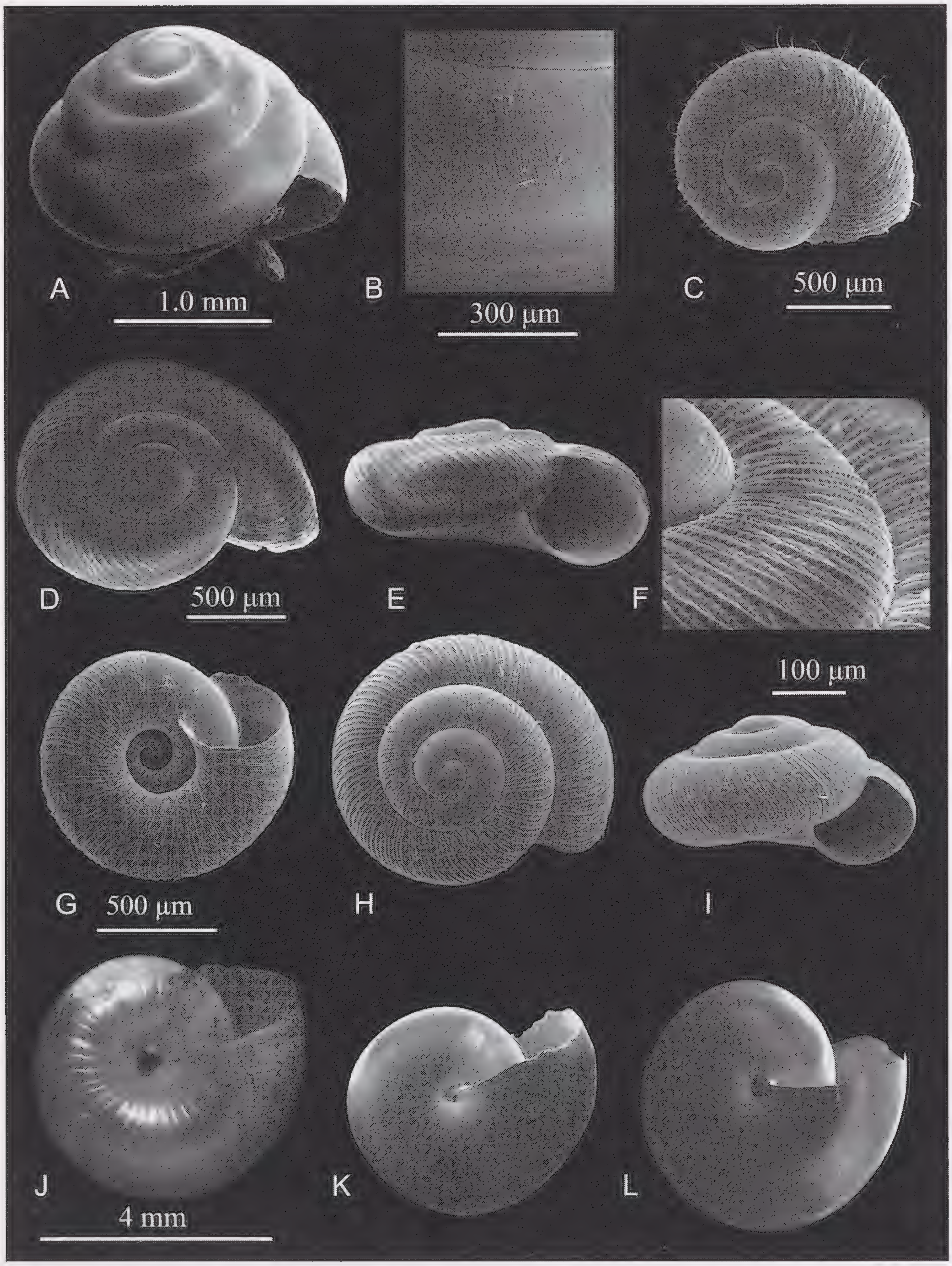

Figure 2. Microsnails with low spires from Sierra de la Madera. **A–B**, *Habroconus trochulinus* (Morelet, 1851) from Rancho San Fernando (site 5). **C**, *Thysanophora hornii* (Gabb, 1866) from Rancho Cieneguita (site 1). **D–F**, *Striatura meridionalis* from Rancho San Fernando (site 5). **G–I**, *Punctum* species from Rancho Cieneguita (site 1). **J**, *Glyphyalinia* cf. *umbilicata* from Rancho Cieneguita (site 1). **K**, *Glyphyalinia indentata* from Gastonia, Gaston County, North Carolina, USA. **L**, *Glyphyalinia umbilicata* from Austin, Travis County, Texas, USA. (Scale bars are as indicated except for bars shared by D and E, and separate bars for rows three and four.)

in the Mexican states of Chihuahua, Nuevo León, Puebla and Veracruz (Bequaert and Miller 1973, Thompson, 2011).

Three *Zonitoides arboreus* (T. Say, 1817 as *Helix*) shells were found in its usual habitat of rotting logs at the microwave antenna (Site 2). These shells have 3–4 whorls, D between 2.67 and 4.59 mm, indistinct growth wrinkles, and minute incised lines along the axis of the whorls. This is the first record of *Z. arboreus* from Sonora; but the species is common over much of North America from Canada to Central America and the Caribbean, and has been introduced into agricultural areas worldwide (Pilsbry 1946). *Zonitoides arboreus* is widespread in Arizona and New Mexico (Bequaert and Miller 1973, Metcalf and Smartt 1997) and occurs in the Mexican states of Chihuahua, Nuevo León, Puebla and San Luis Potosí (Naranjo-García and Fahy 2010).

Family Helicarionidae

Twelve *Habroconus trochulinus* (Morelet, 1851) (Fig. 2A–B) were found alive under leaves in rock crevices along a permanent stream at the base of an east-facing ridge at Rancho San Fernando (Site 5). The single specimen imaged has 4 whorls, D = 2.03 mm, H = 1.64 mm and is similar to all others. This species resembles *Euconulus fulvus* (Müller, 1774) in size and shape but has distinctive microstructure of densely packed, radial striae spaced evenly at intervals of 5 μm and traces of spiral striae near sutures of whorls and on the first whorl. Initial 0.2 whorls are nearly smooth. These specimens extend the range of this species in Mexico from Chiapas, Michoacán, Puebla, and Veracruz (Thompson 2011).

Family Helminthoglyptidae

Five shells of a *Sonorella* Pilsbry, 1900 species were found in talus or piles of rocky debris at four sites. Shells average D = 13.48 mm (range = 13.4–14.3 mm) and have about 4 whorls. Two were immature at death. *Sonorella* has many species distinguishable only by anatomy of the genital complex (W. B. Miller 1967, Bequaert and Miller 1973). Mead *et al.* (2010) list 15 *Sonorella* species from Sonora. The Sierra de la Madera is near the southernmost limit of *Sonorella* and includes the type locality for *Sonorella perhirsuta* (W. B. Miller, 1967).

Identification of Sierra de la Madera *Sonorella* must await collection and dissection of live adults.

Family Philomycidae

The only slug found was a 10–15 mm specimen of *Pallifera arizonensis arizonensis* (Pilsbry, 1917 as *Philomycus* Rafinesque, 1820) in leaf litter at Site 1. The type locality for *P. a. arizonensis* is in the Santa Rita Mountains in Santa Cruz County, Arizona. The subspecies is known from a few localities in Arizona and Sierra Purica in northern Sonora (Bequaert and Miller 1973).

Family Punctidae

Thirty two specimens of a *Punctum* Morse, 1864 species were found in litter in oak woodland at Site 1 (Fig. 2G–I). These snails have D from 0.85–1.32 mm (mean = 1.101, *N* = 30), 2.2–3.5 whorls (mean = 2.94, *N* = 30), and U = 0.200–0.333D (mean = 0.284, *N* = 29) and are the first record of *Punctum* in Sonora. The species name to be applied to these shells is questionable. They most closely resemble figures and descriptions of shells from the Dragoon Mountains of Arizona tentatively called *Punctum californicum* H. A. Pilsbry, 1897 (Pilsbry 1948: 648) but differ from that species in smaller size and more depressed shell (Barry Roth pers. comm. 2011). They are similar to *P. minutissimum* (Lea 1841) in size and closely-spaced major riblets but are more depressed and lack the downturned aperture of that species. They differ from *P. vitreum* H. B. Baker, 1930 in having only 3–4 minor riblets between major riblets. Another widespread species in the American Southwest similar to *P. vitreum* is probably undescribed (Jeff Nekola pers. comm. 2011); but it may be the same as our species and Pilsbry's specimens from the Dragoon Mountains. Further studies of anatomy and DNA will be necessary to provide a final species identification.

Family Succineidae

A broken succineid shell found under a rock at site 1 was H = 6.92 mm , D = 3.91 mm, and WH = 3 with deeply-indented sutures. A badly broken shell was seen and discarded at site 3. Two live specimens with shells about 5 mm long were found in the flood plain of the stream at site 5. These shells had distinctly tapering shells with deeply-incised sutures like some species of *Catinella* Pease, 1871. Dissection found these to be immature and not assignable to species or genus (E. Naranjo-García, pers. comm. 2010). *Succinea luteola luteola* Gould, 1848 and *Succinea luteola sonorensis* Fischer and Crosse, 1878 are known from Sonora.

Family Thysanophoridae

A total of 22 individuals of *Thysanophora horni* (W. M. Gabb, 1866 as *Helix*) were found in litter at four sites. The largest seven individuals average 3.46 whorls (range 2.4–3.8) and D = 3.02 mm (range = 1.6–3.45 mm). Umbilicus diameter averages 0.227D (range = 0.185–0.267D). The species is widespread in Sonora and Mexico (Bequaert and Miller 1973, Hubricht 1985, Naranjo-García and Fahy 2010, Thompson 2011).

Family Valloniidae

Vallonia perspectiva (Sterki, 1893) was the most common snail (*N* = 123) found during the study. These were found at sites 1 and 5. Three adult shells with reflected lips had 2.7–3.2 whorls and D from 1.83–1.97 mm. One specimen has 41 major riblets on the last whorl. *Vallonia perspectiva* is known in Mexico from a few records in Sonora and one in Chihuahua (Bequaert and Miller 1973, Hubricht 1985, Naranjo-García and Fahy 2010). This species is easily distinguished by its size, open umbilicus, and complex shell sculpture.

Family Vertiginidae

Gastrocopta Wollaston, 1878 occurred at most sites. Five of seven species had the transverse basal lamella, deeply places palatal lamellae, and the complexly-fused angular and parietal lamellae of Subgenus *Immersidens* Pilsbry and Ferriss, 1900.

Gastrocopta (*Immersidens*) *ashmuni* (Sterki, 1896) (Fig. 3A) was found at sites 1 (*N* = 12) and 5 (*N* = 4). Shells have 4–4.5 (mean = 4.14) whorls, shell lengths from 1.63–2.19 (mean = 1.85) mm, and shell width of 0.94–1.07 (mean = 1.003) mm. These shells have huge, united angulo-parietal lamellae forming a reversed "y" shape and a distinctive columella that extends onto the parietal wall and appears as an infraparietal. Most of the shells resemble *G. ashmuni* form *minor* (Sterki, 1898) in size, number of whorls, and length of aperture free from shell; but the largest shell is much too large for *G. ashmuni* form *minor.* This species is known from several sites to the north and west in Sonora and in Arizona and New Mexico.

Gastrocopta (*Immersidens*) *cochisensis* (Pilsbry and Ferriss, 1910 as *Bifidaria* Sterki, in Pilsbry,1891) was found in litter at Rancho Cieneguita (site 1) (Fig. 3E). The two intact shells differ from *G. ashmuni* in having smaller lamellae in the aperture and a columella extending only to the junction of the columellar and parietal walls. These shells have 4 whorls, length of 1.90–2.06 mm, and width of 1.05 mm. This species is known in Sonora only from "arroyo 8 km S. Guaymas" (Pilsbry, 1953.) The Guaymas site was listed by Bequaert and Miller (1973) and Thompson (2011) but not by Mead *et al.* (2010).

Snails assignable to *Gastrocopta* (*Immersidens*) *dalliana* (Sterki, 1898) from sites 1, 4, and 5 varied considerably among sites and indicated presence of more than one closely-related species in this limited area. Shells from site 4 (Rancho Mesa Quemada) are quite variable. Five intact shells are similar to most written accounts of this species in their small size

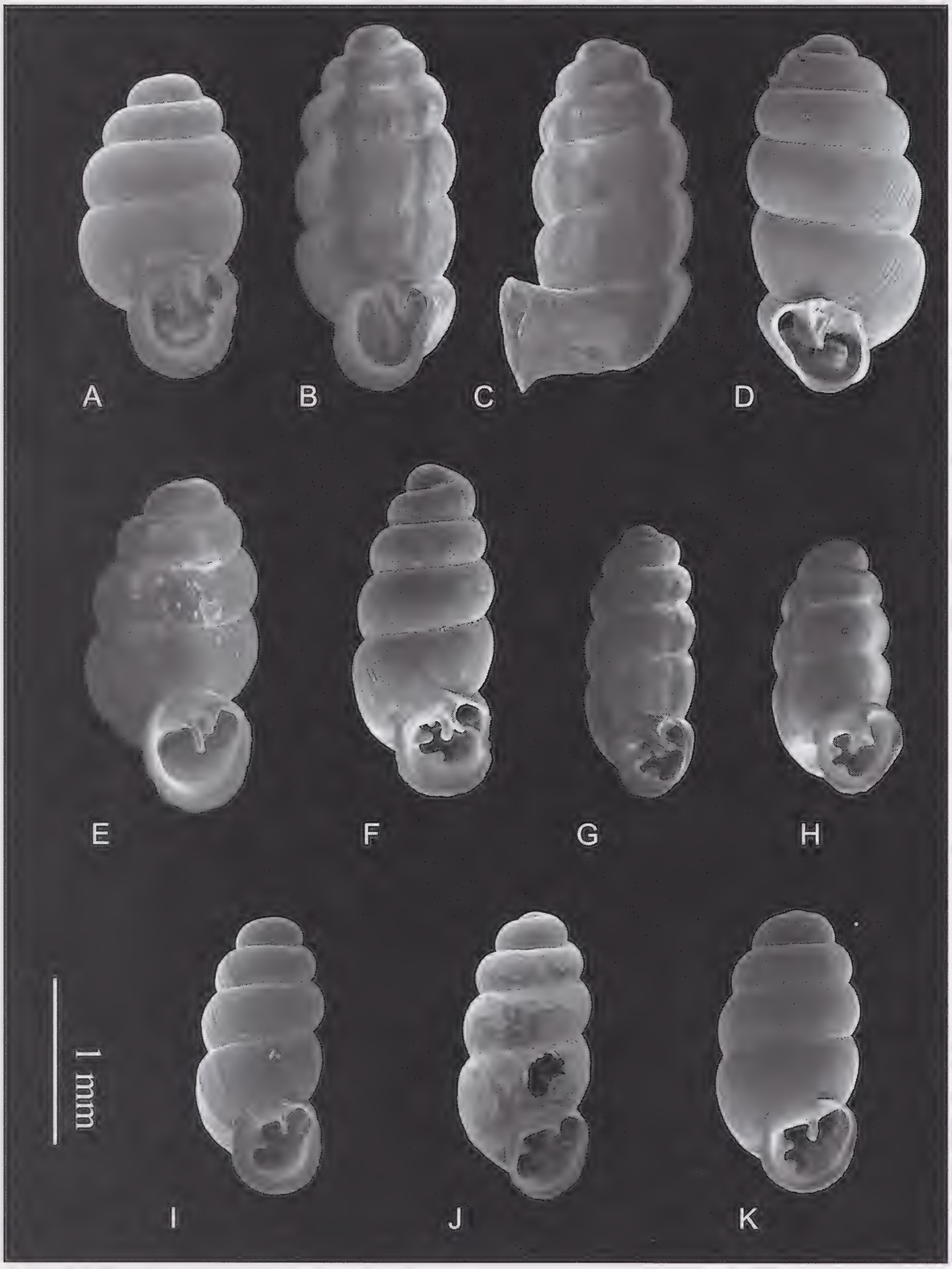

Figure 3. *Gastrocopta* of Sierra de la Madera. **A**, *Gastrocopta ashmuni* from Rancho Cieneguita (site 1). **B–C**, *Gastrocopta perversa* form *sana* from Rancho Mesa Quemada (site 4). **D**, *Gastrocopta perversa* Sterki from Rancho Cieneguita (site 1). **E**, *Gastrocopta cochisensis* from Rancho Cieneguita (site 1). **F**, *Gastrocopta* cf. *dalliana* with infraparietal from Rancho Cieneguita (site 1). **G–H**, *Gastrocopta dalliana* without infraparietal from Rancho Mesa Quemada (site 4). **I–J**, *Gastrocopta pellucida* from Rancho Cieneguita (site 1). **K**, *Gastrocopta pilsbryana* from Rancho Cieneguita (site 1).

(1.64–1.74 mm in length, mean = 1.69 mm, and 0.70–0.79 mm in width, mean = 0.75 mm), aperture shape, and placement of lamellae. Two of these shells (Fig. 3G) are like *G. dalliana dalliana* of Pilsbry (1948: 902, Figure 488) in all respects. Three shells (Fig. 3H) have more complete apertures and a columellar lamella bending downward like Pilsbry's (1948: 902, figure 489) illustration of *G. d. bilamellata* (Sterki and Clapp,1909); which is the larger, western form found over much of Arizona, Sonora, northern Chihuahua and Baja California (Bequaert and Miller 1973). All shells at Rancho Mesa Quemada are smaller than *G. d. bilamellata* and are included in *G. d. dalliana* (Jeff Nekola pers. comm. 2011).

Specimens from higher elevations at site 1 (Rancho Cieneguita) and site 5 (Rancho San Fernando) resemble *Gastrocopta dalliana* with the infraparietal fold as described by Sterki (1898) and ignored by all subsequent authors. These 21 specimens are significantly larger in shell length (1.60–2.06 mm, mean 1.905) and diameter (0.78–0.88 mm, mean 0.832) than *G. dalliana* at Rancho Mesa Quemada (two sample T-test assuming unequal variance). The two species of *G. dalliana*-like snails at Sierra de la Madera seem to represent both the form described by Sterki (1898) and the widely distributed form called *G. dalliana* by all others since 1900. Examination of the type specimens and further studies using DNA and anatomy will be necessary to determine which of these represents a new species.

Three shells of *Gastrocopta perversa* (Sterki, 1898) (Fig. 3D) were found at Rancho Cieneguita (site 1). These sinistral animals are larger than sympatric *G. ashmuni* and have more extended aperture necks. Nine *Gastrocopta perversa* form *sana* (Pilsbry, 1916) (Fig.3B–C) were found at Rancho Mesa Quemada (site 4). The *G. p. sana* shells are similar in size and shape to *G. perversa* at site 1 and noticeably larger than *G. ashmuni* from all sites. Recognition of the larger *G. perversa* is consistent with the presence of two species at each of two sites. Both forms of *G. perversa* are known from other sites in Sonora (Pilsbry 1948, Bequaert and Miller 1973) as *G. ashmuni*.

The subgenus *Gastrocopta* Wollaston 1878 is represented by *Gastrocopta* (*Gastrocopta*) *pellucida* (Pfeiffer,1841 as *Pupa*

Say,1821) found at Rancho Cieneguita (site 1). Three of these are very small and narrow with the last two whorls similar in diameter (Fig. 3I), but a larger one (Fig. 3J) is more tapered, and has a less reflected aperture. The three intact shells averaged WH = 4.2, H = 1.76 mm, and D = 0.817 mm. The species is known from Sonora (Naranjo-García 1991, Mead *et al.* 2010).

The subgenus *Vertigopsis* Sterki 1893 is represented by two *Gastrocopta* (*Vertigopsis*) *pilsbryana* (Sterki, 1890 as *Pupa*) found at site 1. The best specimen had 4.5 whorls, H = 1.81 mm, and D = 0.89 mm (Fig. 3K). This species is known from Sonora (Thompson 2011).

Family Zonitidae

A *Glyphyalinia* Von Martens,1892 species ranked second in abundance with 53 specimens from four sites (Table 1). Many specimens are small and broken, but a sample of 6 large, intact shells average D = 4.12 mm (range = 2.44 – 5.26 mm) with 3.66 whorls (range = 2.7–4.2). Umbilicus averages 6.2% of diameter but ranges from 3.7–9.3% in three adult shells from Rancho Cieneguita. One specimen from Rancho Cieneguita (Fig. 2J) has about 33 incised lines in the last whorl and largest umbilicus in the sample. Two other adults have 29 and 33 incised lines in the final whorl. Shells resemble G. *umbilicata* (Singley in Cockerell, 1893 as *Zonites* Montfort, 1810) from Texas (Fig. 2L) more closely than typical *G. indentata* (T. Say, 1821 as *Helix*) from Gaston County, North Carolina (Fig. 2K) and are assigned to that species. *Glyphyalinia indentata* ranges from southeastern Canada, over much of the U.S. and Mexico south to Guatemala (Metcalf and Smartt 1997). It is reported in Sonora, Nuevo León, Tamaulipas, and San Luis Potosí as *Glyphyalinia indentata indentata* (Naranjo-García and Fahy 2010) and in Puebla and Mexico near Teotihuacan as *G. indentata paucilirata* (Morelet, 1851). *Glyphyalinia indentata* probably comprises a complex of several anatomically-distinct species (Hubricht 1985). Snails in the Sierra de la Madera are certainly not *Glyphyalinia indentata* of the eastern U.S. (Fig. 2K) and probably do not represent *G. indentata paucilirata* (Bequaert and Miller 1973, Metcalf and Smartt 1997). The wider umbilicus of our specimens suggests affinity with *Glyphyalinia umbilicata* from Texas (Fig. 2L), which is currently recognized for many U. S. populations previously referred to as *G. paucilirata* (Turgeon *et al.* 1998). Further anatomical and DNA work on this group will undoubtedly reorganize this genus dramatically.

DISCUSSION

Sierra de la Madera is a "sky island" (Heald 1951, Warshall 1995) of mesic environment surrounded by xeric lowlands and has many microhabitats within a relatively small area. The fauna of the Sierra de la Madera is derived from diverse sources. Seven species (*Striatura meridionalis, Zonitoides arboreus, Glyphyalinia umbilicata, Gastrocopta pilsbryana, Punctum* species, *Succinea* species, *and Vallonia perspectiva*) represent widespread Nearctic lineages, while four species (*Habroconus trochulinus, Pallifera arizonensis, Thysanophora horni*, and *Gastrocopta pellucida*) are clearly Neotropical. The six forms in *Gastrocopta* (*Immersidens*) are members of autochthonous radiations in the region but are derived from a Neotropical lineage. The *Sonorella* species is also autochthonous but cannot easily be assigned to any biogeographical source as the family is widespread in the New World. The snail fauna is similar to several floras (McLaughlin 1955) in having both northern and southern biogeographic affinities, but seems to include more local and regional endemic species. Very little is known about biogeography, habitat requirements, species' anatomy or fossil record of land snail faunas worldwide (Metcalf and Smartt 1997). This situation seems especially true for Sonora (Naranjo-García 1991, Mead *et al.* 2010). Snails from the Sierra de la Madera are consistent with the Southwestern Molluscan Province discussed by Bequaert and Miller (1973); but analyses of additional faunas in this region will be necessary to elucidate its biogeographical history. While Mead *et al.* (2010) listed 59 species, 27 genera, and 17 families from Sonora, there are few reports of actual faunal assemblages for the state. The diversity of this fauna is rather meager compared to Mexican states with better-studied faunas (Thompson 2011) or the somewhat larger Arizona (Bequaert and Miller 1973). Our collection of 18 total species (about 1/3 of the total known for the state) and 4 taxa new for the state in a relatively short time and limited area suggests that many more species remain unrecorded. We expect the list of Sonoran snail species to grow considerably with additional sampling.

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RESEARCH NOTE

Radular variations in freshwater snails of the family Lymnaeidae (Mollusca: Gastropoda: Basommatophora) from northwestern Iran

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Abstract: The Family Lymnaeidae is comprised of large aquatic snails that are intermediate hosts of parasitic flukes. In the present study, radular morphology, teeth length, and tooth formula of field-collected lymnaeid snails including *Galba truncatula* (Müller, 1774), *Lymnaea stagnalis* (Linnaeus, 1758), *Radix auricularia* (Linnaeus, 1758), and *Radix gedrosiana* (Annandale and Prashad, 1919) were investigated. The conical and dextral freshwater snails were collected from their natural habitats in West Azerbaijan province, northwestern Iran, in fall 2010. The snails were rinsed, measured, and taxonomically identified. The radula of all specimens was extracted and stained using Mallory II. Besides the morphological variations, the mean length of radular teeth significantly increased with the body size of the examined lymnaeid snails. Morphometric investigation indicated that the mean length of lateral (L) (427.86 ± 6.9 μm) and marginal (M) (427.82 ± 6.86 μm) radular teeth of *Lymnaea stagnalis* differed significantly from those of other examined lymnaeid snails. The average number of radular M teeth and L teeth were the highest in *R. auricularia* and *L. stagnalis*, respectively. The specimens with smaller body size had the most transverse teeth rows (*G. truncatula*, 115.6 ± 3.7 μm). It was concluded that radular teeth morphology and morphometry within lymnaeid species could be one of the character sets for classification of lymnaeid snails.

Key words: systemic, flukes, morphology, Mallory II stain

The phylum Gastropoda includes most molluscs and the subclass Pulmonata is comprised of mainly freshwater and land snails with about 28,000 species worldwide (Morton 1979). Molluscs of the genus *Lymnaea* Lamarck, 1799 (*Galba* Schrank, 1803/*Radix* Montfort, 1810) act as intermediate hosts of the common liver flukes (Shubkin *et al.* 1992, Rognlie 1994). So far, seven species of this genus have been identified in Iran (Mansourian 1986). Of those, *Radix gedrosiana* (Annandale and Prashad, 1919) is more widely distributed throughout the country (Mansourian 1986, Imani Baran *et al.* 2011).

The radula is a dynamic structure in most molluscs which consists of a long ribbon of tissue with repeated rows of teeth along its length (Rumam and Thornton 1967). This part of snails' buccal mass is therefore a key feature for discriminating gastropods. In lymnaeid snails, the morphological identification of *Galba truncatula* (Müller, 1774) based on shell features is not very difficult, while in other lymnaeid snails in the genus *Radix* differentiation of snails based on morphological features is very difficult except for the aquatic snail *Radix auricularia* (Linnaeus, 1758) (Mas-Coma 2005, Pfenninger *et al.* 2006). The morphology of the radular teeth, including the number of teeth rows and teeth per half row has often been used for differentiating species of lymnaeid snails. The pattern and number of teeth in a row can also be used in identification as they are different in each species (Burch 1982). In the present study radular variations including length of teeth and tooth formula of lymnaeid snails from northwestern Iran was investigated.

Colonies of conical and dextral freshwater snails were collected from bodies of water located in West Azerbaijan province (37°33′10.08″ N, 45°04′33.24″ E), northwestern Iran, during Fall 2010. Snails with shells larger than 4 mm in length were collected from each habitat for a period of 30 min. Live specimens were carried to the Malacology laboratory and rinsed in distilled water. Before dissection, the length of the shell (the maximum distance from the apex to the base of the aperture) was measured. Lymnaeid snails were taxonomically identified according to keys provided by Paraense (1976, 1984), Mansourian (1986), and Pfleger (1999) with a magnifying glass.

A total of 488 specimens were examined using optic microscopy and then photographed. For this purpose, whole snails were placed in a 90°C water bath for a minimum of 5 minutes and then the buccal mass including radula was completely removed (Kristensen 1984). The radula was put into the 7.5% KOH overnight and stained by Mallory II (Mansourian 1986). The cleaned radula was mounted with a mounting medium (Canada Balsam). The radular teeth located in central (C), lateral (L), and marginal (M) parts were measured using an Olympus compound microscope (400× and 1000×) with an ocular micrometer. To determine the size of radular teeth, the length of each tooth from the apical cusp to the base was considered. The number of transverse rows and teeth in each row were counted and recorded to determine tooth formula.

The radular measurements were analyzed by SPSS statistical program (version 14, SPSS Inc., Chicago, Illinois, USA) using two sample *t*-test. Probability of < 0.05 was regarded as significant.

The morphology of the radula has been considered as one of the most commonly used sources of information for studying molluscan systematics. The shape and form of molluscan radular teeth are typically unique to a genus or species (Pfleger 1999). The radula in lymnaeid snails is classified as Pulmonate radula with a quantity of minute teeth arranged in transverse and longitudinal rows with one central tooth, a few lateral teeth, and multiple marginal teeth (Mansourian 1986, Pfleger 1999).

The radular pattern, considered as the adult type of the species, appeared in all studied specimens. However, morphological differences were observed in the radular teeth (Figs. 1A–D). The C tooth in the middle of each row of the radula was triangular with specific cusp shape. The L teeth were also triangular but different in size. In *Radix gedrosiana* and *Galba truncatula*, the cusp of the L teeth was folded, while it was straight ahead in both *Lymnaea stagnalis* (Linnaeus, 1758) and *Radix auricularia*. The M teeth were filamentous in all specimens with an enlarged basal part except for *Galba truncatula*. Radular teeth (C, L, and M) were smaller and thinner in *Galba truncatula* than other examined lymnaeid snails.

The lengths of radular teeth increased in the C tooth of larger snails (*Lymnaea stagnalis*, 551.2 ± 40.17 μm) ($P < 0.05$) (Table 1). The smallest sizes of radular teeth were recorded in *Galba truncatula* ($P < 0.05$). Furthermore the radular teeth formula was found to be specific for each lymnaeid snail. Besides the variation in teeth size and formula, lymnaeid snails with larger shell sizes had larger teeth sizes. There was significant relationship between shell length and teeth size within each species ($P = 0.0001$) (Table 1).

Table 1 shows the correlation between the number of transverse rows and the size of the examined snails. *Galba truncatula* (body size < 10 mm) had the highest numbers of teeth rows (115.6 ± 3.7) among examined lymnaeid snails ($P = 0.0001$). The average numbers of L teeth were approximately the same among the specimens except for *Lymnaea stagnalis* (13 ± 2) (Table 1). There was a significant increase in the number of radular L teeth in the snails with larger body sizes ($P = 0.0001$). In *Radix auricularia*, the average numbers of radular M teeth was the highest (22 ± 2) among examined lymnaeid snails ($P < 0.05$) (Table 1).

In molluscan systematics, morphological variations and teeth arrangement in the radula are common sources of information to discriminate and identify species or genera. Thus intraspecific variations in radular characteristics are important (Malek 1962, Pfleger 1999, DeMaintenon 2004). In the present study, radular morphology findings could be one of the character sets for classification of lymnaeid snails in northwestern Iran and other parts of the country. C tooth morphological features varied within the lymnaeid species with cuspate center and enlarged

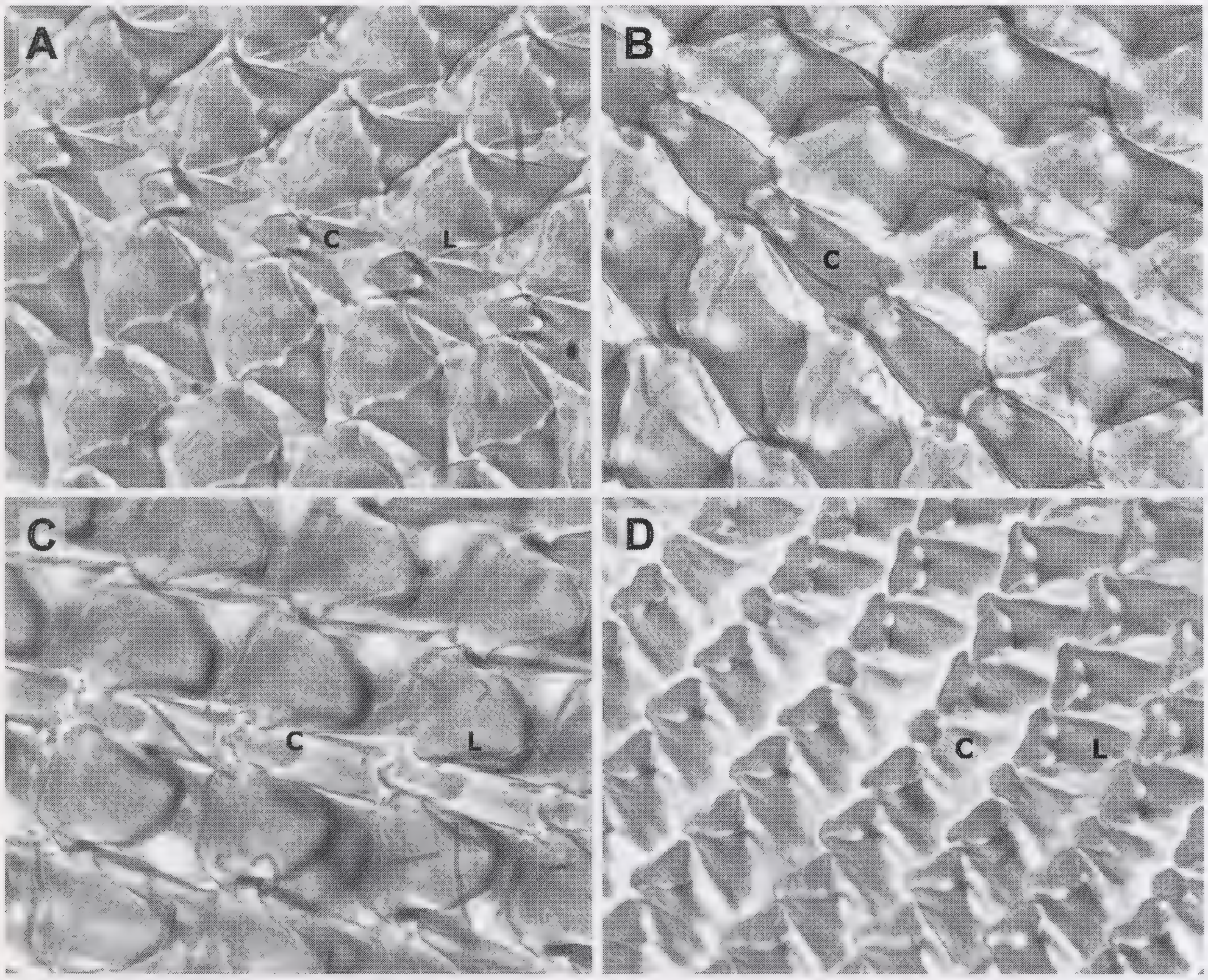

Figure 1. The radular central (C) and lateral (L) teeth of lymnaeid species (**A**, *Radix auricularia* 1000×; **B**, *Radix gedrosiana* 1000×; **C**, *Lymnaea stagnalis* 1000×; **D**, *Galba truncatula*. 1000×). All using Mallory II staining.

Table 1. Means and standard deviation (Mean ± *SD*) for radular teeth measurements and numbers from lymnaeid snails radulae ($N = 488$). Abbreviations: C, central tooth; L, lateral teeth, M, marginal teeth, and *SD*, standard deviation.

Lymnaeid snail species	Shell length (mm)	No. of measured radulae	Radular teeth length (µm)			No. of radular teeth rows	No. of radular teeth		Radular teeth formula
			C	L	M		L	M	
Radix auricularia[a]	19.33 ± 3.11	122	237.3 ± 6.33	283.62 ± 13.48	283.6 ± 13.49	98.6 ± 2.3[e]	8 ± 1	22 ± 2[i]	30.1.30
Radix gedrosiana[b]	16.27 ± 4.14	122	282.38 ± 42.62	246.38 ± 43.18	246.35 ± 43.22	99.1 ± 2.9[f]	9 ± 1	17 ± 3	26.1.26
Lymnaea stagnalis[c]	35 ± 4.34	122	551.2 ± 40.17	427.86 ± 6.9	427.82 ± 6.86	95.1 ± 2.1[g]	13 ± 2[j]	14 ± 2	27.1.27
Galba truncatula[d]	7.33 ± 1.01	122	163.01 ± 15.26	125.01 ± 18.12	124.99 ± 18.13	115.6 ± 3.7[h]	9 ± 2	11 ± 1	20.1.20
Total		488							

t tests: [a+b] (C, L, M: $P > 0.05$), [a+c] (C, L, M: $P = 0.001$), [a+d] (C: $P > 0.05$, L, M: $P < 0.05$), [b+c] (C: $P = 0.001$, L, M: $P > 0.05$), [b+d] (C, L, M: $P = 0.001$), [c+d] (C, L, M: $P = 0.001$), [g] ($P = 0.0001$), [e, f, h] ($P > 0.05$); [i, j]: $P < 0.05$.

base. In addition, each L and M tooth had a folded cusp with one to three cusps along the posterior edge and larger at the end. The average number of radular teeth and rows elucidated that there was a significant increase between the number of rows and lymnaeid snail shell sizes. The intraspecific variability related to the radular formula has been previously reported in opisthobranch snails (Bertsch 1976), *Triopha* sp. (Berg, 1880) (Ferreira 1977), *Euplica varians* (Sowerby, G.B.I, 1832), *E. versicolor* (Sowerby, 1832) (DeMaintenon 2004), and *Polycera aurantiomarginata* (García and Bobo, 1984) (Martinez-Pita *et al.* 2006). Nearly all researchers described a direct correlation between the body size and number of radular rows in specimens larger than 4 mm (Ferreira 1977, Ocaňa *et al.* 2004). In the present study *Galba truncatula* with an average shell size less than 10 mm had the greatest number of radular rows. This finding was in agreement with Ferreira (1977) and Ocaňa *et al.* (2004) who noted that the number of radular rows was higher in specimens smaller than 10 mm. The average number of radular L teeth was greater in specimens with larger shell sizes. The number of radular M teeth was the highest in specimens with the lowest average number of radular L teeth (*Radix auricularia*). In addition, shell length and teeth sizes within each species had a significant correlation. These findings indicated that radular formula and teeth sizes may be useful criteria besides snails' morphology and shell sizes for accurate discrimination of lymnaeid snails in the region. For instance, the radular feature of *Lymnaea columella* (Say, 1817) with 95 transverse rows of teeth including eight L teeth and 22 M teeth were indicative in Brazil (Paraense 1983). It was concluded that the length of the radular teeth increases with the body size of the specimens and lymnaeid snails smaller than 10 mm have the most teeth rows. In addition, radular morphology within lymnaeid species may be systemically informative.

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RESEARCH NOTE

False spike, *Quadrula mitchelli* (Bivalvia: Unionidae), is not extinct: First account of a live population in over 30 years

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Abstract. During a recent survey a small population of *Quadrula mitchelli* (Simpson, 1895), a species thought to have been extinct, was discovered in Texas. In total, 7 live individuals were collected from the Guadalupe River near Gonzales, Gonzales County, Texas. Our finding represents the only known population for this species in Texas and the first record of live specimens in over 30 y, which is significant because this species is currently under review for protection under the Endangered Species Act.

Key words: freshwater mussels, unionids, Texas, threatened, rare species

Quadrula mitchelli (Simpson, 1895), false spike, is a rare central- and west- Texas endemic unionid mussel species that historically inhabited the Rio Grande, Guadalupe, San Antonio, Colorado and Brazos River basins. In central Texas, living specimens have not been observed since the late 1970s, and in the Rio Grande drainage recent accounts come from subfossil and fossil specimens (Howells 2003). Until recently, the only evidence that this species was not extinct came from a recent collection of a fresh-dead individual from the San Saba River, a major tributary of the Colorado River (Randklev *et al.* in press).

During a recent survey in October 2011, an apparently small population of *Quadrula mitchelli* represented by 7 live individuals (Fig. 1) was discovered in the Guadalupe River near Gonzales, Gonzales County, Texas. All of the individuals collected are consistent with taxonomic descriptions provided by Howells (2010). Juveniles were not observed at the site, but given that we used timed tactile and visual searches their presence cannot be ruled out. Shell length ranged from 47.0–54.6 mm, with a median length of 50.6 mm. Other species were collected at the same locality, including *Amblema plicata* (Say, 1817), threeridge; *Cyrtonaias tampicoensis* (I. Lea, 1838), Tampico pearlymussel; *Lampsilis teres* (Rafinesque, 1820), yellow sandshell; *Lampsilis hydiana* (I. Lea, 1838), Louisiana fatmucket; *Megalonaias nervosa* (Rafinesque, 1820), washboard; *Pyganodon grandis* (Say, 1829), giant floater; *Quadrula aurea* (I. Lea, 1859), golden orb; *Quadrula petrina* (Gould, 1855), Texas pimpleback; and *Toxolasma texasense* (I. Lea, 1857), Texas lilliput. Of these species, *Q. aurea* and *Q. petrina* are considered to be state threatened and candidates for protection under the Endangered Species Act (TPWD 2009, USFWS 2011).

The site where these individuals were collected is characterized by steep banks with a small riparian buffer. The adjacent land uses are a golf course and range land. Water velocity and depth were not measured, but appeared to be lower than normal because of the ongoing exceptional drought within the region. *Quadrula mitchelli* was collected primarily along the upstream edge of a point gravel bar in substrate consisting of gravel, coarse sand, and mud. Water current at this locality was slow to moderate and depth was less than 30 cm. A single individual was found at the head of a riffle, partially buried in gravel and sand substrate.

Our finding represents the only known population for this species in Texas and the first record of live specimens in over 30 y (Howells 2010). This find is significant because this species is currently under review for protection under the Endangered Species Act (USFWS 2009). Because of this, a more thorough study of this reach of the Guadalupe River

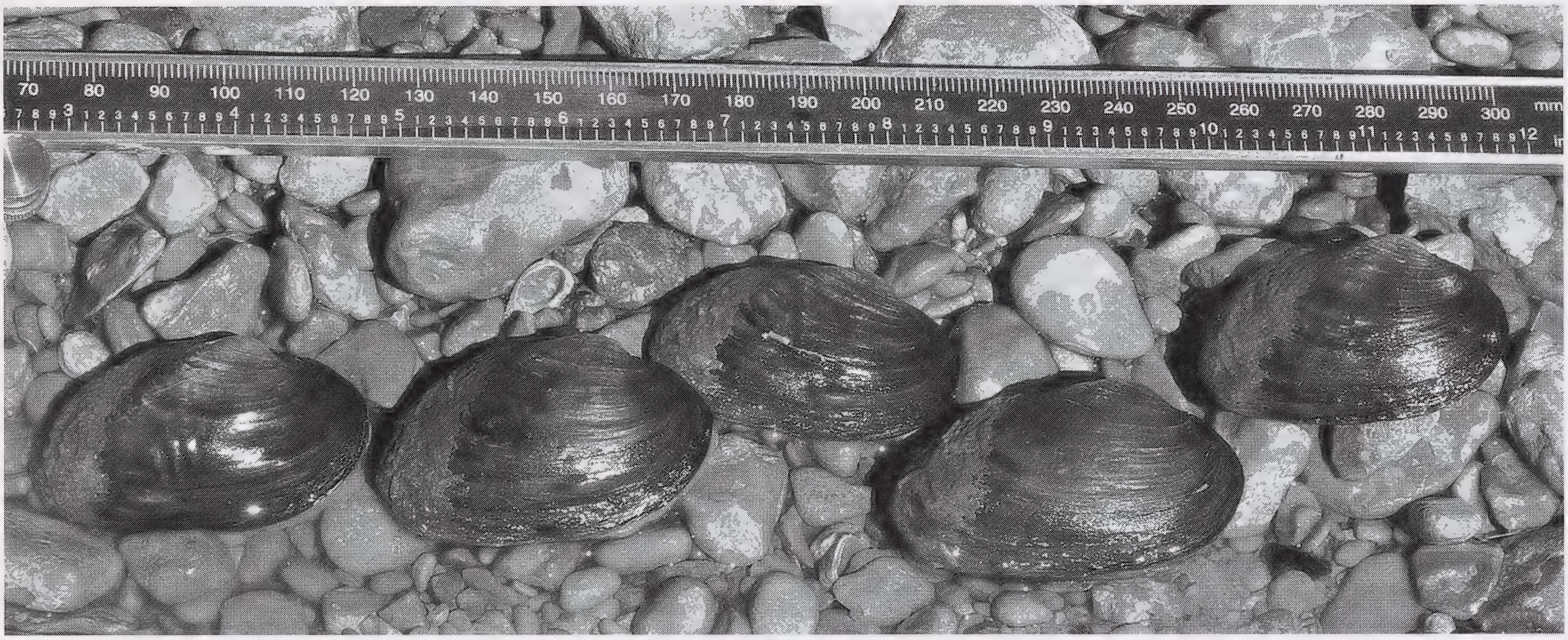

Figure 1. Live *Quadrula mitchelli* collected from the Guadalupe River, Gonzales County, Texas, in 2011.

and its tributaries, such as the San Marcos River is needed to determine the extent of its distribution within this drainage. Additionally, locating a surviving population of *Quadrula mitchelli* creates the potential to study the species' genetics, basic life history, reproductive biology, and habitat requirements, which are unknown or poorly understood.

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RESEARCH NOTE

Glochidial morphology of two species of the genus *Schistodesmus* (Bivalvia: Unionidae) from Lake Poyang, China

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Abstract: Glochidial morphology of two unionid species of the genus *Schistodesmus* Simpson, 1900 from Lake Poyang (China), *Schistodesmus lampreyanus* (Baird and Adams, 1867) and *S. spinosus* Simpson, 1900 were studied for the first time under light and scanning electron microscopy. Glochidial characters, including shape, size, hook, sensory hair tufts and exterior minute sculpture, are presented and compared with some allied species for the identification of glochidia. *Schistodesmus* has small and ovate subtriangular glochidia with styliform hook. Glochidia of the two species of *Schistodesmus* Simpson, 1900 differed from each other in relative abundance of microstylets and micropoints, and valve sculpture.

Key words: glochidium, *Schistodesmus lampreyanus*, *Schistodesmus spinosus*, ultrastructure

Glochidia are highly specialized parasitic larvae of freshwater unionoids, which develop early in the marsupia of female mussels, then attach to specific fish hosts before becoming free-living juveniles (Lefevre and Curtis 1910). Glochidial morphology has been studied under both light microscopy and scanning electron microscopy (SEM) by many malacologists (Rand and Wiles 1982, Clarke 1985, Kwon and Park 1993, Park and Kwon 1993, Pekkarinen and Englund 1995, Hoggarth 1999). Many morphological characters of glochidia have been used for species identification and phylogenetic analyses of higher taxa (Simpson 1900, Parodiz and Bonetto 1963, Hoeh *et al.* 2001, Graf and Cummings 2006, Pimpão *et al.* 2012).

The fauna of freshwater mussels is diverse in China with about 15 genera and 140 recorded species (Liu *et al.* 1979). However, the current classification of Chinese unionids is mainly based on adult shell features and little is known about soft-anatomical characters (Wu 1998). As for the characters of glochidia, the current information is still inadequate, although glochidia of 19 unionids have been described (Wei *et al.* 1994, Wu 1998). Moreover, the minute shell structures of most glochidia have been neglected. Our present study is a morphological survey of the glochidia of two unionids from Lake Poyang which have never been described before, with the purpose to improve our knowledge and provide more morphological information for phylogenetic analyses of Unionidae.

Gravid females of the two examined species, *Schistodesmus lampreyanus* (Baird and Adams, 1867) and *S. spinosus* Simpson, 1900, were collected from December 2000–May 2001 from Lake Poyang, Jiangxi Province, China (Fig. 1). Adults were identified according to Simpson (1914), Haas (1969) and Liu *et al.* (1979). Voucher specimens of adults and glochidia are deposited at the Institute of Life Science, Nanchang University, Nanchang, China.

Mature glochidia were collected from the demibranchs of gravid females and fixed in 75% ethanol for investigation under light microscopy. Measurements of shell length, shell height and hinge length of 50 glochidia per species were taken under a light microscope equipped with a calibrated micrometer ocular. Mature glochidia of two species, *Schistodesmus lampreyanus* and *S. spinosus*, were taken only from one gravid female each.

For SEM, specimens were fixed for 2 h in glutaraldehyde, dehydrated in a graded ethanol series (30 min each at 30%, 50%, 70%, 90%, 95%, and three washes in 100%) and transferred to acetone for 30 min. Then they were critical-point dried with liquid CO_2 and coated with gold. Observations were made in a Hitachi S-570 SEM at accelerating voltages of 20–30 kV. Terms of glochidial morphology follow Hoggarth (1999).

Unionidae Rafinesque, 1820

The unionid fauna of China is highly diverse with about 140 species recorded (Liu *et al.* 1979), which comprise about

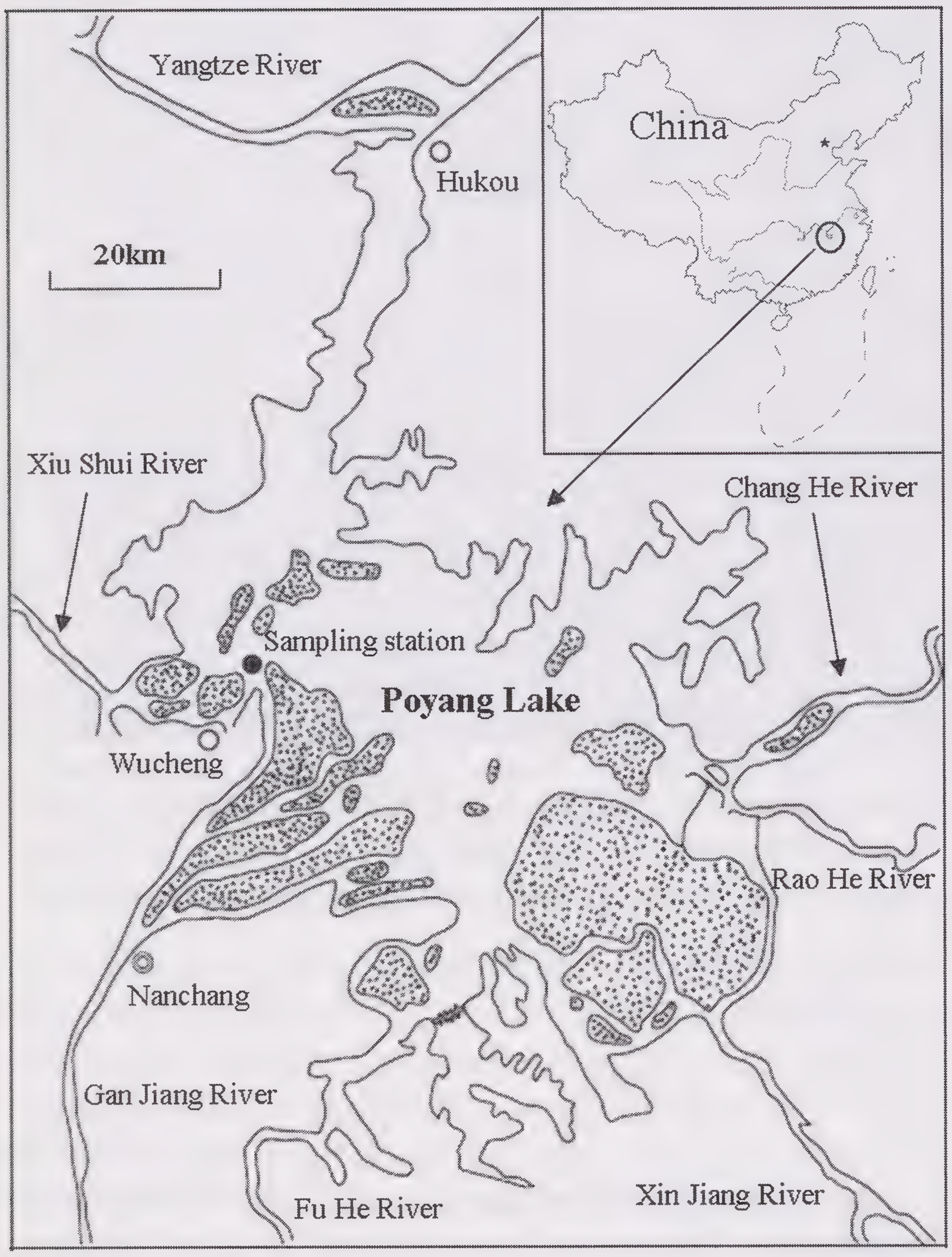

Figure 1. Sampling station in Lake Poyang, China.

18% of world's unionid fauna (Graf and Cummings 2007). About 20% of genera (e.g. *Acuticosta* Simpson, 1900, *Arconaia* Conrad, 1865, *Lepidodesma* Simpson, 1896, *Diaurora* Cockerell, 1903, *Pseudobaphia* Simpson, 1900 and *Schistodesmus* Simpson, 1900) and 70% of species are endemic to China (Liu *et al.* 1979, Prozorova *et al.* 2005, Graf and Cummings 2007).

Genus *Schistodesmus* Simpson, 1900

Diagnosis: Shell medium- or small-sized, triangular, inflated, solid; beaks inflated, elevated above hinge line, located subcentrally or anteriorly; shell surface generally with prominent, concentric ridges. Short-term brooding (tachtictia). Marsupia ectobranchous, with complete septa. Glochidia small-sized, subtriangular, hooked, micropoints only on ventral margin of valve, not extending to hinge.

Type species: *Schistodesmus lampreyanus* (Baird and Adams, 1867).

Schistodesmus lampreyanus (Baird and Adams, 1867)

Glochidium ovate subtriangular (Fig. 2A), nearly symmetric, moderately inflated, length 136.2–156.0 µm (148.3 ± 5.3 µm), height 145.6–167.4 µm (159.7 ± 5.0 µm). Hinge straight, length 104.0–124.8 µm (114.3 ± 4.8 µm). Styliform hook arising as a broad biconcave triangular plate from ventral margin of valve, bluntly rounded at distal end. About 20 microstylets sparsely but uniformly distributed on hook (Fig. 2B). Micropoints occurring sparsely on ventral margin of valve, but not extending to hinge (Fig. 2C), and lateral surfaces of hook, leaving narrow unsculptured distal hook margin. Four pairs of sensory hair tufts formed by hairs of different lengths, one close to hinge, and three others located near ventral margin of valve, and near hook, arranged in triangular shape (Fig. 2D). Exterior surface of valve coarsely pitted throughout, with numerous white granules (Fig. 2E). Under high magnification (10,000 x), loose-looped sculpture covering exterior surface of valve (Fig. 2F) can be observed.

Remarks: The glochidia recovered from the marsupia were free and had no residue of the egg membrane, which indicates they had fully developed. Therefore, the bluntly rounded tip of the hook is the mature form, and not an intermediate stage of a pointed tip. The glochidium can be easily distinguished from others in the subfamily Unioninae by its shape, its relative sparse microstylets and micropoints, and its arrangement of micropoints (not extending to the hinge may (?) be a unique feature in Unionidae). It resembles the glochidium of *Unio douglasiae* (Gray, 1833) in the shape of hook and glochidial size (Table 1), but does not share other similarities (Wei *et al.* 1994, Wu 1998).

Schistodesmus spinosus Simpson, 1900

Glochidium ovate subtriangular (Fig. 3A), nearly symmetric, moderately inflated, length 132.4–151.6 µm (143.2 ± 4.2 µm), height 148.9–171.2 µm (164.1 ± 4.2 µm). Hinge straight, length 114.7–128.3 µm (124.6 ± 3.2 µm). Hook styliform, bluntly rounded at distal end (Fig. 3B). Microstylets densely and uniformly distributed on hook. Dense micropoints only on ventral margin of front half of valve (Fig. 3C), and also on lateral surfaces of hook except distal margin (Fig. 3B). Sensory hair tufts in four pairs, grouped (Fig. 3D), identical to those of *Schistodesmus lampreyanus*. Exterior surface of valve sparsely but deeply pitted, dense vermiculate sculptures tightly covering exterior surface of valve (Fig. 3E, F).

Remarks: This glochidium resembles that of *Schistodesmus lampreyanus* in shell outline and hook structure. However, it can be distinguished from the latter by its relative slender shape, its relative dense microstylets and micropoints, and valve sculpture (Table 1).

Taxonomic or phylogenetic studies on the Chinese Unionidae to date are sparse and relied heavily on adult shell features (Simpson 1900, Haas 1969, Liu *et al.* 1979, but see Wu 1998, Huang *et al.* 2002). The classification system currently used by Chinese malacologists mainly followed

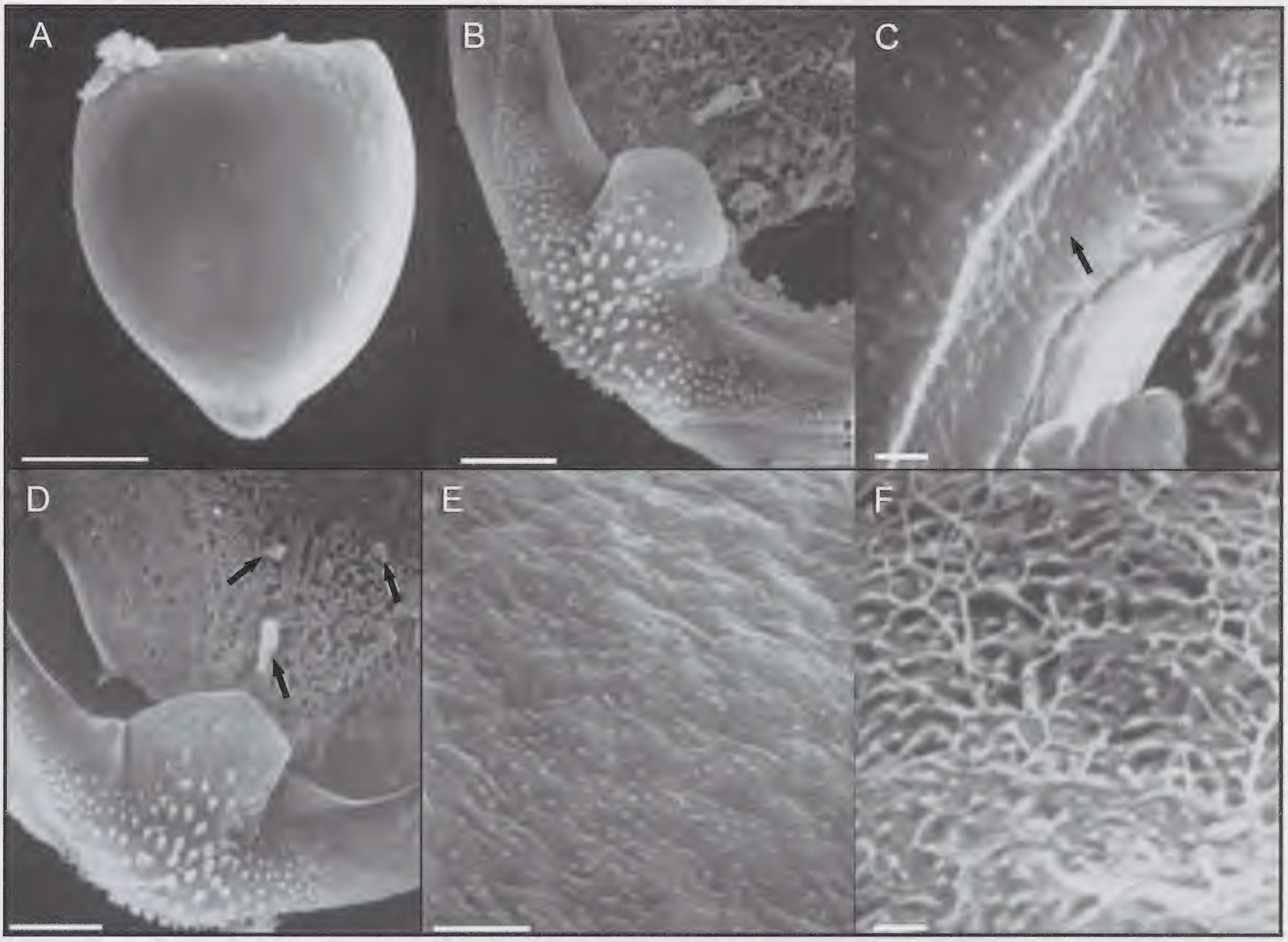

Figure 2. Glochidium of *Schistodesmus lampreyanus*. **A**, exterior valve; **B**, hook with microstylets; **C**, micropoints on the lateral margin of the valve; **D**, sensory hair tufts; **E**, **F**, exterior valve sculpture. Scale bars: A = 50 µm; B, D = 10 µm; C, F = 1 µm; E = 5 µm.

Haas's (1969) global revision of Unionoida, and divided into two subfamilies, Unioninae and Anodontinae. Graf and Cummings (2006) made the latest global revision on classification of Unionoida with a combination of DNA and morphological data. The Unionidae were divided into two subfamilies, Unioninae and Ambleminae, the traditional Anodontinae were placed as part of the Tribe Anodontini within Unioninae, while the Old World amblemines were either assigned to Unioninae or placed as *incertae sedis* assemblages within Unionidae. But most Old World genera previously assigned to the Ambleminae (e.g. Huang *et al.* 2002) were not included in their phylogenetic analysis due to insufficient taxon overlap.

Schistodesmus is endemic to China and traditionally assigned to the subfamily Unioninae (Liu *et al.* 1979, Wu 1998) or Tribe Unionini within Unioninae (Graf and Cummings 2006). The taxon is represented only by two species in China, *S. lampreyanus* and *S. spinosus*. Glochidial characters support the placement of *Schistodesmus* in the subfamily Unioninae. However, three characters of the glochidium (relative small size, valve outline and arrangement of micropoints on the ventral rim of valve) make it quite different from those of other genera in the same subfamily, which would provide some information for unionid taxonomy. Unfortunately, there is little knowledge about its population ecology and reproductive biology, although the two species are all listed as threatened (Shu *et al.* 2009). Therefore, more comprehensive studies on its conservation measures are urgently needed.

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Table 1. Glochidial features of three unionid species from Lake Poyang, China

	*Unio douglasiae**	*Schistodesmus lampreyanus*	*Schistodesmus spinosus*
Shell shape	broadly triangular	ovate subtriangular	ovate subtriangular
Gln#	0.020	0.024	0.023
L/H ratio	1.050	0.929	0.873
Hook			
Shape	Styliform, blunt in the distal	Styliform, bluntly rounded in the distal	Styliform, bluntly rounded in the distal
Microstylets	dense, uniformly distributed	sparse, uniformly distributed	dense, uniformly distributed
Micropoints	dense, extending to hinge	sparse, not extending to hinge	dense, not extending to hinge
Sensory hair turfs	four pairs	four pairs	four pairs
Exterior surface of valve	shallow pits, sculpture consisting of fine non-overlapping lines	shallow pits, loose-looped sculpture	deep pits, vermiculate sculpture

Note: # Gln (glochidial index) = Length (L) × Height (H)
* Data from Wu (1998)

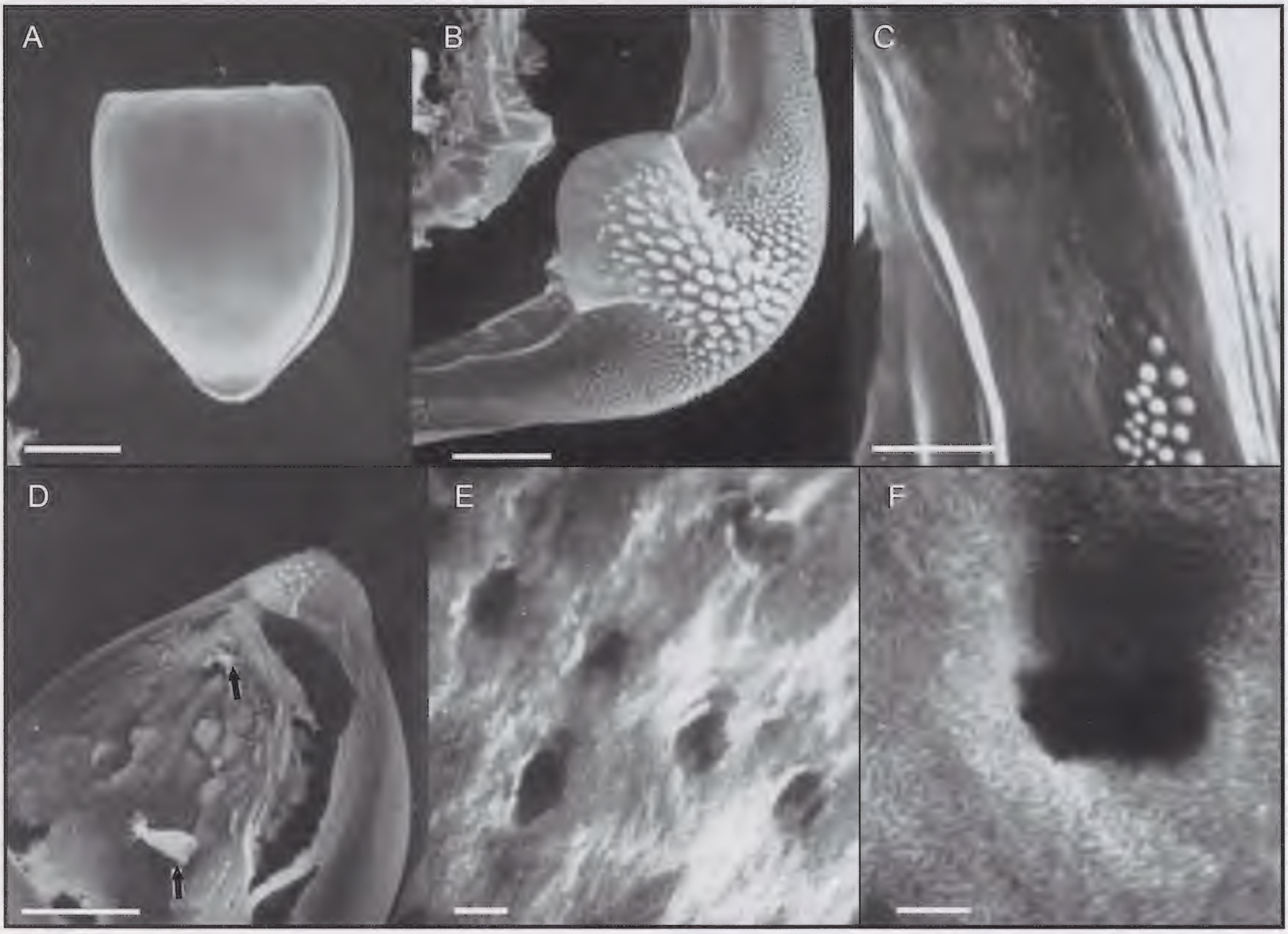

Figure 3. Glochidium of *Schistodesmus spinosus*. **A**, exterior valve; **B**, hook with microstylets; **C**, micropoints on the lateral margin of the valve; **D**, sensory hair tufts; **E**, **F**, exterior valve sculpture. Scale bars: A = 50 µm; B = 10 µm; C = 3 µm; D = 30 µm; E, F = 1 µm.

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RESEARCH NOTE

In situ behavioral observations of the mesopelagic squid *Stigmatoteuthis dofleini* (Cephalopoda: Histioteuthidae)

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Abstract: *In situ* observations of the cock-eyed squid *Stigmatoteuthis dofleini* (Pfeffer, 1912) were recorded in high definition video at 439 m depth off the eastern seaboard of Japan during a survey by the ROV *HyperDolphin*. Novel behaviors including a quick body turn (ratcheting) were observed and are reported here with discussion on their ecological significance.

Key words: cephalopod, histioteuthid, behavior, submersible, Japan

Midwater cephalopods are a significant link in the ecology of midwater and deepwater systems. Although it is assumed that evolutionary pressures shape the behaviors and physiognomy of deep-water forms as in other ecosystems, there is little information that might allow us to understand how behaviors could influence trophic ecology in deep water.

The squid genus *Stigmatoteuthis* (Pfeffer, 1900) is a member of the family Histioteuthidae, commonly found in midwater zones. These squid are frequent prey items of deep diving predators such as the Sperm Whale, *Physeter macrocephalus* (Clark 1977). Histioteuthids are perhaps best known for their eyes, which differ in shapes between the sides of the head, their relatively tiny fins, and their ample photophore coverage. These anatomical differences from other squids must impact the behavioral abilities and, therefore, the ecology of histioteuthids, although precisely how is unknown due to limited observations of live specimens.

We present here a brief description of a single mature female *Stigmatoteuthis dofleini* (Pfeffer, 1912) specimen observed with a submersible. Notes are provided on its behavior, including a previously undescribed twisting maneuver that we have called "ratcheting". We discuss the implications of the behaviors observed for histioteuthid ecology as compared with other mesopelagic cephalopods. It is only by recording the natural behaviors and abilities of deep-sea species that we can begin to understand how their unique morphology and behavior relates to their ecology.

A transect that included Oyashio-derived cold waters, Kuroshio-derived warm waters, and their respective mixing and frontal zones was conducted along the eastern seaboard of Japan using the R/V *Kaiyo* from 20 April–6 May 2002. Water masses were identified using satellite imagery and XCTD (eXpendable Conductivity Temperature Depth) probes, and dive surveys using the ROV *HyperDolphin* were conducted to determine the vertical distributions of the macroplanktonic gelatinous organisms (Lindsay *et al.* 2004, Lindsay *et al.* 2008). The ROV *HyperDolphin* was equipped with a high-definition camera integrating an ultra sensitive super HARP (High gain Avalanche Rushing Photo-conductor) tube and illumination was provided by five 400 W SeaArc HMI/MSR metal halide lamps. Detailed information on the camera and lighting specifications has been reported elsewhere (Lindsay *et al.* 2008). During ROV *HyperDolphin* Dive 107 on 3 May 2002 (36°39.6′N, 142°06′E), a large individual of the cock-eyed squid *Stigmatoteuthis dofleini* was recorded on video at 439 m depth (10:10 AM) and subsequently captured. Video footage was recorded continuously and simultaneously with depth/time overlay on Sony BCT-D124L Digital Beta Cam tapes via an analogue composite signal, and without overlay on BCT-124HDL HDCAM tapes via a native digital signal at 1080i and 30 frames per second.

Physico-chemical data were collected using a SeaBird SBE19 CTD and an SBE43 oxygen sensor attached to the vehicle. The specimen was collected for positive identification using a suction sampler. A motor-driven fan slurped the specimen and the water surrounding it into a 12 cm-diameter intake hose and then into a single clear acrylic canister of 30 cm diameter. It was transferred into a photo tank and still digital photographs of the specimen were taken with a Nikon D1H digital camera with a macro lens (AF Micro Nikkor 105 mm

1:2.8 D) and recorded in TIFF-RGB format at an image size of 2000 × 1312 pixels. Illumination was provided by National Ref Lamp color-balanced flood bulbs (PRF-500WB) that lit the specimen from the side against a black felt backdrop.

According to Young and Vecchione (2008), this species has also been known by the names *Histioteuthis hoylei* Goodrich, 1896, *H. dofleini* Pfeffer, 1912, and *H. arcturi* Robson, 1948. Young and Vecchione (2008) distinguished a northern and southern species in this group, and identified them both as *Stigmatoteuthis.* Due to the limited information on the group, we believe a description of the specimen herein is valuable.

The specimen was a mature female with a total length of 33.5 cm and a dorsal mantle length (DML) of 8 cm. Inter-pupil distance was 4.5 cm. Tentacle lengths were 19 cm. The fin insertion began 3 cm from mantle apex and continued to the tip of the mantle. Arm lengths along the right side of the body were as follows: Arm I = 13 cm, Arm II = 13.5 cm, Arm III = 15.5 cm, Arm IV = 12 cm. Photophore pattern around the eyes, arms and anterior mantle match those described by Young and Vecchione (2008) as *S. hoylei.* Compound photophores with reflecting chambers and red filters as described by Young and Vecchione (2007) were also present. The specimen is housed at JAMSTEC, sample reference number HD107ss1c-048425.

The animal first appeared on camera at 10:02:02 AM. Its body was oriented obliquely head down, with arm pairs I and II curled dorsally over the head so that the distal tips nearly touched the mantle apex. Arm pair III was curled similarly but the tips curled dorsally to the midpoint of the body, near the mantle edge. Arm pair IV was slightly curled in the same direction with the tips extending more downward. The tentacles were draped over the 4th arms such that the distal portion hung vertically from the midpoint of the 4th arms. The left side of the body with the enlarged left eye was facing the side with the ROV but oriented upwards towards the surface.

After 1 min 12 s of exposure to the lights of the ROV, the squid rolled its arms down, forming the arms and tentacles into a tight cone. This behavior took 11 seconds to complete (see http://www.jamstec.go.jp/biogeos/j/mbrp/deco/StigmatoVideo1.html). The long axis of the animal was oriented at an angle approximately 80° from horizontal. The skin on the arms and mantle was highly reflective and appeared shiny in the video, with the photophores appearing as distinct dark spots throughout the body. This appearance was very different than submersible observations of *Histioteuthis heteropsis* (Berry, 1913), which appears dark or red in color, with little reflectivity and photophores that are smaller and white (Hunt, pers. obs.)

The squid appeared to hover in place, neither diving nor ascending for 39 s. Next, the animal turned its body 90° so that the ventral surface was facing the camera and neither eye was pointed directly toward the lights. The way in which this turn occurred was novel and we refer to it here as "ratcheting." First, the mantle alone quickly turned about its longitudinal axis approximately 45° clockwise (if viewed from above looking down at the apex of the mantle). The head, arms and tentacles then rotated quickly in the same direction, while the mantle simultaneously rotated the opposite direction (back to its original position). The mantle section then rotated again, following the twisting head and arm complex until the entire body had been turned about 90°. It appears that the considerably larger and heavier head and arm complex get more rotational thrust by the countering movement of the lighter mantle section (see http://www.jamstec.go.jp/biogeos/j/mbrp/deco/StigmatoVideo2.html).

With the ventral side now facing the camera, the motion of both fins could be observed. The squid maintained its depth in the water using its fins to hover. Each fin created two waves simultaneously, with one wave beginning at the apex of the mantle traveling anteriorly along a fin, and the other wave beginning at the anterior margin of the fin (fin insertion point) and traveling towards the apex. The two waves met near the center of each fin, canceling each other out, while also counterbalancing the motion in the opposite fin (see http://www.jamstec.go.jp/biogeos/j/mbrp/deco/StigmatoVideo3.html). This behavior continued for 3 min 8 s, during which a camera close up for 43 s allowed us to count the fin beat cycles and respiratory cycles (measured by the closing movements of the funnel). The squid beat its fins 52 times in 43 s (1.2 cycles/s) and it respired 27 times over 43 s (0.6 cycles/s). During this time, the squid dove 0.5 m (1.2 cm/s).

At 10:07:21 the fin motion abruptly changed in frequency and mode of beating. The animal began diving more rapidly. Now, each fin exhibited only one wave at a time, and the wave moved completely from the anterior edge of the fins to the posterior edge. Fin waves were synchronized to provide propulsive force downward. This behavior continued for 2 min 21 s until specimen collection began. Another ratchet maneuver was recorded, this time in the opposite direction to the first ratcheting and only resulted in a 45° body turn. A camera close up during this faster diving allowed fin beat cycles and respiratory cycles to be measured over 25 s. The squid beat its fins 50 times (2.0 beats/s) and respired 17 times (0.7 cycles/s). It dove 1.7 m during this time (6.8 cm/s).

At 10:09:42 the ROV suction sampler arm touched the squid. It showed a strong thigmotactic response, rapidly extending its arms in all directions. The strong flow pulled in most of the squid, but three arms grabbed the outside of the funnel on the suction sampler. One of the three arms quickly lost its grip in the strong flow (3 s). The other two arms held the animal in place for 16 s before letting go, despite the considerable flow within the collection tube observed on the video (though not directly measured).

The taxonomy of histioteuthids has received more attention than most deep-sea squid families. An overview of the current status of the genus is given by Young and Vecchione (2008). They placed all members of the *hoylei* group into the genus *Stigmatoteuthis*, but they noted that the Pacific specimens had been divided into a more temperate northern Pacific species (*S. dofleini*) and a southern Pacific species (*S. hoylei*). The two species are distinguished primarily on geographical differences. We have identified our specimen based on its morphology and its capture location as *S. dofleini* in keeping with this treatment.

The positioning of histioteuthids in the water column and its relationship to the differences in the eyes has been the subject of some speculation. Voss (1967) suggested that the animal hunted above the bottom, with the large eye facing down and small eye facing up. Bitjukova and Zuev (1976) demonstrated that the small eye is adapted to see better at great depths while the larger eye is more functional at shallower depths. Hunt (1996) reported that *Histioteuthis heteropsis* Berry, 1913) was found repeatedly in a stereotypical posture when first approached. The specimen of *Stigmatoteuthis dofleini* in this study exhibited a very similar posture (described above) to the stereotyped pose described by Hunt (1996) when first approached. A photo showing a similar pose has also been presented online by Young and Vecchione (2007) and in print by Church (1971) and Vecchione (2001).

Histioteuthids are commonly found as part of the diet of large carnivores such as deep-diving whales (Okutani *et al.* 1976; Clarke and Goodall 1994), sharks (Vaske Junior *et al.* 2009), swordfish (Young *et al.* 2006), and other squid (Parry 2006). Such predation pressure must influence the evolutionary ecology of histioteuthids as in any other prey group. Yet histioteuthids do not have the same defensive abilities as more energetic squid species. For example, the fins are proportionately small and ill-suited for sustained, vigorous swimming. Their fins have not been observed to be used as hydrofoils (Hunt, pers. obs.), as has been observed in other squids (Hunt *et al.* 2000). Their eye differences must alter their visual abilities as compared to other squids, both for hunting and anti-predatory responses. When disturbed by submersibles, histioteuthids rarely ink or attempt rapid jet escape (Hunt, pers. obs.). When inking does occur, it is in diffuse clouds that bathe the animal and not the tight pseudomorphs associated with rapid escape (Hunt 1996). We suggest that histioteuthids rely on primary (pre-attack) anti-predatory defenses rather than secondary (post-attack) defenses commonly observed in other squids (e.g. protean behavior, misdirection, using ink as a pseudomorph).

Consequently, histioteuthids should benefit from adaptations that allow them to remain undetected, such as holding still and not stirring bioluminescence in the water that would give away their position. Remaining still would be aided by their strong ability to counter-illuminate, an ability suggested by the structure and placement of their photophores (Young 1977). When histioteuthids do move, they do so in a way that minimizes water disturbance. Their small fins turn the body slowly, preventing bioluminescent displays from being triggered in the surrounding water. The pivoting ability of the photophores allows a more precise control of bioluminescent light as the animal changes attitude in the water column. The ratcheting movement described here was clearly well-controlled and purposeful to the animal. Histioteuthids are typically situated with the eyes (each with its unique abilities) facing opposite directions. The ratcheting behavior is a quick way to spin the body around and change the direction each eye is facing, thereby taking advantage of the different abilities of each eye. Broadly dispersed ink (when it does occur) may provide the same kinds of chemical defenses as it does in other squids, but not the same kinds of visual deterrence (i.e. pseudomorphs). The eyes differ in size, color, and orientation. We note that some cephalopods can see and use polarized light to hunt (Shashar *et al.* 1998, 2000). We suggest future work examine the large upward-pointed eye for its ability to detect polarized light.

The use of submersibles to gain information on natural behaviors has proponents and critics. We agree with Vecchione and Roper (1991) who stated, "If what we are seeing are defensive postures, then at least we know what defensive postures look like." It is more likely that animals use a natural behavior from their repertoire than invent a completely new response to a novel threat (Hunt 1996). Furthermore, information gleaned from direct observation of animals *in situ* can provide insights into the evolutionary ecology of poorly-known species. Like all animals, deep-sea animals such as *Stigmatoteuthis* have a range of behaviors adapted for their habitat. We can only hope to understand these animals after we observe what we can of their natural behaviors.

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We sincerely thank the captain and crew of the R/V *Kaiyo* as well as the commander Y. Yoda and the operations team of the ROV *HyperDolphin* for their dedicated efforts. We are indebted to the anonymous reviewers of this manuscript for their efforts in improving this manuscript.

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In Memoriam

Allen, J. Frances (1916–2011; U.S.A.)
Andrews, Jean (1923–2010; U.S.A.)
Athearn, Herbert David (1923–2011; U.S.A.)
Athearn, Nadine (1927–2011; U.S.A.)
Baker, John Allen (1920–2011; U.S.A.)
Bosch, Donald Taeke (1917–2012; Oman/U.S.A.)
Callomon, John (1928–2010; Germany/U.K.)
Clarkson, Peter S. (1960–2011; Australia)
Coomans, Hendrikus [Henry] Eduard (1929–2010; Netherlands)
Crawford, George Ivor (1910–2011; U.K.)
DuPont, John Eleuthère (1938–2010; U.S.A.)
Frassinetti Cabezas, Daniel Ángel (1939–2010; Chile)
Golikov, Aleksandr Nikolaevich (1931–2010; Russia)
Haven, Dexter Stearns (1918–2011; U.S.A.)
Hemmen, Jens (19**–2012; Germany)
Johnson, Paul (19**–2012; U.K.)
Joosse, Joos (1930–2010; Netherlands)
Kawano, Toshie (1938–2010; Brazil)
Krolopp, Endre (1935–2010; Hungary)
Kuiper, Johannes Gijsbertus Jacobus [Hans] (1914–2011; Netherlands)
Linden, Jan van der (1938–2011; Netherlands)
Lucum, Lois (19**–2011; U.S.A.)
Mateokin, Petr (1917–2010; Russia)
Meeuse, A. D. J. (1914–2010; Java/Netherlands)
Morton, John Edward (1923–2011; New Zealand)
Paget, Oliver E. (1922–2011; Austria)
Paraense, Wladimir Lobato (1914–2012; Brazil)
Roberts, Trevor Digby (1916–2011; U.S.A.)
Sánchez, Teresa María (1945–2011; Spain)
Schander, Carl Frederic Christoffer (1960–2012; Sweden)
Schneider, William T. (1930–2011; U.S.A.)
Sorbi, Enrico (1941–2011; Italy)
Stein, Carol Belle (1937–2010; U.S.A.)
Suduiraut, Emmanuel Guillot de (1938–2010; France/Philippines)
Tripp, Jessie [Jay] Jauchius (1923–2010; U.S.A.)
Vervoort, Willem (1917–2010; Netherlands)
Watkins, Rodney M. (1949–2010; U.S.A./U.K.)
Wimbleton, P. T. (1938–2010; U.K.)

Partial list of colleagues who have died since 2010 (Coan *et al.*, 2012), supplemented by access to the working document for the next edition. We thank Gene Coan and Alan Kabat for their advice and access to their unpublished material.

Coan, E. V., A. R. Kabat and R. E. Petit. 2012. 2,400 years of Malacology, 9th ed., March 8, 2012. American Malacological Society: http://www.malacological.org/publications/2400_malacology.php.

Christoffer Schander, 1960–2012

Christoffer Schander was born in 1960 in Borås, Sweden. After a too short but very productive life in malacology and marine invertebrate zoology, Chris sadly and unexpectedly passed away in Bergen, Norway, on February 21, 2012. The world has been deprived of one of its most energetic and broadly-based malacologists, and one of the few systematists of Aplacophora.

Chris was characterized by imagination, a broad experience in marine invertebrate zoology and a fine sense of fun and empathy. His early experiences were as a Rotary exchange student to the U.S., a writer of science fiction, and a Captain in the Swedish Naval Reserve. He was accepted as a graduate student at Gothenburg University in 1990, where research in zoology was honed by a rigorous education culminating in a Ph.D. degree (1997). As a graduate student, he held a number of assistantships in research and teaching.

Chris wrote that his goals in research were "to understand the role that evolutionary forces and phylogeny have played in organism diversity." More than 60 percent of his ~90 publications are concerned with Mollusca, particularly Aplacophora and gastropod Pyramidellidae, but including Bivalvia, Scaphopoda, Polyplacophora, and terrestrial slugs. However, all invertebrates were grist to his mill of more generalized papers on molecular phylogeny and phylogenetic taxonomy, ecology, barcoding, nomenclature, biogeography, conservation, and molecular genetics. His delight was taking part in faunal surveys.

In 1998 Chris arrived at the Woods Hole Oceanographic Institution as a postdoctoral fellow on an NSF grant "Partnerships for Enhancing Expertise in Taxonomy" (PEET) for studies on the deep-sea Aplacophora. Following a stint as Chief Scientist at the Danish University Arctic Station, Qeqertarsuaq (Disco), Greenland, Chris obtained a position as Professor of Marine Biodiversity at the University of Bergen, also becoming thematic leader at the Center of Excellence in Geobiology. Last year he spent a sabbatical year at Auburn University to increase his knowledge of phylogeographic and phylogenomic methods in exploring the faunal evolutionary history of Norwegian hydrothermal vents and seeps. On his return to Bergen, he became Director of the University Museum.

Chris's enthusiasm was infectious. Whenever the subject was around biology, Chris would be miles ahead leading us to look at the broader implications. He was generous with his time. When on-board a research vessel to collect all sorts of marine invertebrates, particularly in the fjords and vents off Norway, he was never happier (photo).

Besides teaching and research, Chris expended much time for funding to make Bergen a center of excellence for marine research, now being carried on by his colleagues in Bergen, the U.S., Europe, and Russia. To this end, the Christoffer Schander Memorial Fund at the University of Bergen has been established to honor his memory and to continue his eager support of cooperation and scientific exchange between individuals and institutions. Donations to the fund in the U.S. may be made through http://gump.auburn.edu/halanych/For_Chris, and in Europe through: http://www.uib.no/rg/mb/schander-fund/about-the-schander-memorial-fund.

Amélie Scheltema, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.

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American Malacological Society Treasurer's Report

Calendar Year 2010

Submitted July 27, 2011

At year end (December 31, 2010), funds available to the Treasurer were as follows:

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Vanguard Total Stock Portfolio #85/9888588127	$20,428.60	
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Vanguard Total Stock Portfolio #85/9888590542	$62,634.12	
Vanguard Bond Index Fund #84/98885881217	$75,697.67	
TOTAL INVESTED ASSETS	$158,760.39	
TOTAL ASSETS: Operating & Endowment Funds		**$181,694.96**

Total assets rose in 2010 from $172,636.10 to $181,694.96, an increase of $9,058.86.

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Scope. The *American Malacological Bulletin* is the scientific publication of the American Malacological Society and serves as an outlet for reporting notable contributions in malacological research. Manuscripts concerning any aspect of original, unpublished research, important short reports, and detailed reviews dealing with molluscs will be considered for publication.

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Citation Format.

Beattie, J. H., K. K. Chew, and W. K. Hershberger. 1980. Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during summer mortality. *Proceedings of the National Shellfisheries Association* **70:** 184–189.

Hillis, D. M. 1989. Genetic consequences of partial self fertilization on populations of *Liguus fasciatus* (Mollusca: Pulmonata: Bulimulidae). *American Malacological Bulletin* **7:** 7–12.

Seed, R. 1980. Shell growth and form in the Bivalvia. *In:* D. C. Rhoads and R. A. Lutz, eds., *Skeletal Growth of Aquatic Organisms.* Plenum Press, New York, New York. Pp. 23–67.

Yonge, C. M. and T. E. Thompson. 1976. *Living Marine Molluscs.* William Collins Son and Co., Ltd., London.

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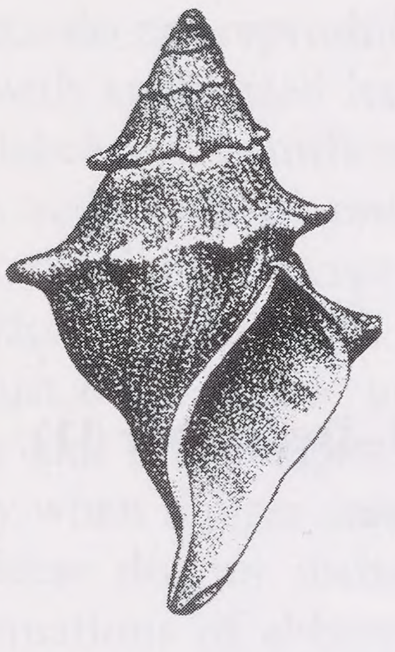

The American Malacological Society
2013 Annual Meeting

The 79th meeting of the American Malacological Society (AMS) will be held jointly with the 18th International Congress of Unitas Malacologica (UM) from July 21st to July 28th, 2013 in Ponta Delgada on the island of São Miguel, Azores, Portugal. The program will include thematic symposia, open contributed sessions, poster sessions, field trips, and social events.

Information on meeting registration, schedule, accommodations, and submission of titles for talks and abstracts will soon be posted at UM (http://www.unitasmalacologica.org) and AMS (http://www.malacological.org) websites. Contact Dr. Peter Marko (pmarko@clemson.edu) for further information and inquiries about sessions and symposia related to AMS participation.

Travel funds for AMS student members will be available. Information about student travel grants will be forthcoming on the AMS website.

The Azores is an archipelago comprised of nine volcanic islands located in the North Atlantic Ocean, 1500 km west of Lisbon and 1900 km east of Newfoundland. São Miguel is the largest island in the archipelago and is the location of an international airport (PDL) accessible from North America with direct flights from Boston and Toronto as well as from several European cities. Ecotourism is abundant in the Azores, particularly activities focused on the flora, fauna, and geothermal features of the archipelago.

We hope to see you there!

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